GENETIC VARIABILITY AND CHARACTER ASSOCIATION OF AMARO COFFEE (*Coffea arabica* L.*)* ACCESSIONS AT AWADA, SOUTHERN ETHIOPIA

MSc THESIS

BY

DESALEGN ALEMAYEHU

OCTOBER, 2018

JIMMA, ETHIOPIA

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A thesis

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We, the undersigned, member of the Board of Examiners of the final open defense by **Desalegn Alemayehu** have read and evaluated his/her thesis entitled **"Genetic Variability and Character Association of Coffee (*Coffea. arabica* L.) at Awada Accessions Southern Ethiopia"** and examined the candidate. This is therefore to certify that the thesis has been accepted in partial fulfillment of the requirements for the degree Master of Science in **Plant Breeding**

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DEDICATION

I dedicate this piece of work to my lovely parents, Alemayehu Kidane and Zewditu Worku**.**

STATEMENT OF THE AUTHOR

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LIST OF ACRONOMYAND ABBREVIATIONS

ANOVA	Analysis of Variance
CBD	Coffee Berry Disease
CLR	Coffee Leaf Rust
CSA	Central Statistical Agency
EBI	Ethiopian Biodiversity Institute
EIAR	Ethiopian Institute of Agricultural Research
FAO	Food and Agricultural Organization
GAM	Genetic Advance as percent of Mean
GCV	Genotypic Coefficient of Variation
ICO	International Coffee Organization
IPGRI	International Plant Genetic Resource Institute
JARC	Jimma Agricultural Research Center
PCA	Principal Component Analysis
PCV	Phenotypic Coefficient of Variation
SAS	Statistical Analysis Software
SNNPR	Southern Nations Nationalities People's Region
USAID	United States Agency for International Development

BIOGRAPHICAL SKETCH

The author, Desalegn Alemayehu, was born in March 1991 at Girar Jarso District, North Shewa Zone of the Oromia Regional National State in Central Ethiopia. He attended his elementary education at Abdi Saga Elementary School from 1998- 2006 and his Secondary School at Fitche High School from 2007-2008. The author attended his preparatory school at Fitche Senior Secondary school from 2009-2010. After successful completion of Ethiopian Higher Education Entrance Qualification Certificate Examination in 2010, he joined Madawalabu University in 2011 and graduated in 2013 with a Bachelor of Science in Plant Science. He was employed since May 2014 by Ethiopian Agricultural Research Inistitute (EIAR), Jimma Agricultural Research Center (JARC) and served as junior researcher in crop research till September 2016. Then he joined the postgraduate program of Jimma University to pursue his MSc degree in plant breeding.

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ABSTRACT

Fifty eight Amaro coffee (Coffea arabica L.) accessions and six standard check were evaluated for genetic variability and character association at Awada Agricultural Research Sub-Center, Southern Ethiopia using morphological traits. The experiment was laid out in an 8x8 simple lattice design with eight coffee accessions per each incomplete block. Analysis of variance for 19 quantitative characters revealed significant difference (P<0.05) among the accessions in coffee bean yield, plant height, height up to first primary branch, main stem diameter, canopy diameter, number of bearing primary branches, fruit width, fruit length, bean thickness, bean width, leaf width, 100-coffee beans weight, coffee berry disease and coffee leaf, average inter nodes length of main stem, length of first longest primary branch, number of primary branches, bean length, leaf size. High phenotypic and genotypic coefficient of variation was recorded for coffee bean yield, coffee berry disease and coffee leaf rust disease severity. Genotypic coefficients of variation were very close to their corresponding estimates of phenotypic coefficient of variation suggesting greater role of the genotype in the expression of these traits. High estimates of heritability and genetic advance as percent of mean observed for coffee berry disease, coffee leaf rust and bean yield. Coffee yield has positive and significant genotypic association with number of primary branches (rg=0.704), number of bearing primary branch (rg=0.613), number of main stem node (rg=0.619), stem diameter (rg=0.335) and canopy diameter (rg=0.376), whereas average inter node length of main stem (1.083), number of main stem nodes (0.427), canopy diameter (0.414), height up to first primary branch (0.300) and number of bearing primary branch (0.294) had maximum direct effect on yield. Cluster means analysis revealed appreciable variation for quantitative characters. The distances between most of these clusters were highly significant at (P<0.01), suggesting the possibility of getting genetically divergent accessions for hybridization. The first six principal components exhibited more than one Eigen value and accounted for 77.7% of the total variation. The first two principal components with values of 23.32% and 18.85%, contributed more to the total variation. Shannon- diversity indices for the traits fruit shape, young leaf tip color and growth habit were high. This indicates that these qualitative traits contributed more to genetic variation in this study. Coefficient of variation, heritability estimates, correlation analysis, path analysis and multivariate analysis confirmed presence of variation among tested accessions. However, additional traits of interest should be studied over year and locations including physiological, quality and biochemical analysis with the support of advanced molecular techniques.

Keywords: *Correlation, Heritability, Path coefficient analysis, Clustering, Principal component*

1 INTRODUCTION

Coffee belongs to the family *Rubiaceae* and to the genus *Coffea* (Berthaud and Charrier, 1988; Coste, 1992). *Rubiaceae* has over 6000 species and 500 genera (ITC, 2002). Of these, the most economically important genus is *Coffea* (Wellman, 1961), comprising of about 124 species (Davis *et al*., 2011). *Coffea arabica* Linnaeus and *Coffea canephora* Pierre are the two most widely cultivated species in the World, in which the former accounts for about 61% of world production (ICO, 2018). Arabica coffee is predominantly grown in the tropical and subtropical regions of the world (Berthaud and Charrier, 1988; Davis *et al*., 2012). Arabica coffee (*Coffea arabica* L.) is the only allotetraploid species (2n=4x=44) (Lashermes *et al*., 1999), whereas the rest are diploid and self-incompatible with the exception of *Coffea hetrocalyx* and *Coffea anthonyi* (Nowak *et al*., 2012).

Ethiopia is the homeland and center of genetic diversity of Arabica coffee in the highlands of southwestern and southeastern parts (Vavilov, 1951; Sylvain 1955). The entire genetic diversity of indigenous (wild) Arabica coffee is confined mainly in the afromontane rain forest located in the west and east of Great Rift Valley (Kassahun *et al.*, 2008). The crop is mainly produced in the Southern, South Western and Eastern parts of the country. The total area coverage of coffee in Ethiopia is estimated to be around 700,474.69 ha, of which about 95% is produced by 4 million small scale farmers (CSA, 2017) whereas the estimated annual national production of coffee is about 7.83 millions of 60kg bags (CSA, 2017)**.** The average national production was about 670 kg ha^{-1} (CSA, 2017).

Coffee is crucial to the economies of world's least developed countries, accounting for more than 50% of their exports (ICO, 2015). Coffee is known to be one of the most important beverages in the world (Labouisse *et al.,* 2008). More than 125 million people in the world, derive their income directly or indirectly from its products in cultivation, processing, trading, transportation and marketing (Lashermes *et al.*, 2011; Mishra and Slater, 2012; Gray *et al*., 2013). Arabica coffee *(Coffea arabica)* is one of the world's most valuable agricultural commodities which ranks second after oil in international trade (Geromel *et al*., 2006). Ethiopia is the most important coffee-producing and exporting country in Africa and the sixth most important coffee producer worldwide (ICO, 2018; USDA, 2018).

Different research findings illustrate the importance of the Ethiopian coffee genetic materials in breeding programs for high productivity and disease resistance (Labouisse *et al.*, 2008). Ethiopian *C. arabica* accessions have been used as parents and crossed with commercial varieties to obtain strong hybrid vigor, resulting in higher productivity, excellent quality standard, and partial resistance to CBD and rust in the F1 hybrids in Central America (Chrisophe *et al.*, 2014). Jima Agricultural Research Center has developed and released 40 new coffee cultivars (34 pure lines and 6 hybrids) for different localities having a character of high yielding, resistant to diseases and possesses acceptable quality profile (Tadesse, 2017). However, the production and productivity of coffee in Ethiopia is very low as compared to Brazil. Brazil's coffee production (millions of 60-kg bag) is 56.76 and 51.00 in 2016 and 2017, respectively; while Ethiopia's coffee production (millions of 60-kg bag) is 7.30 and 7.65 in the year 2016 and 2017, respectively (ICO, 2018).

Different researchers have repeatedly reported major contributing factors for such low yields such as lack of improved coffee varieties for different agro ecology with high yield, disease resistance, and best quality; limited availability and adoption of improved coffee cultivars and lack of well characterized and distinctly variable breeding materials readily available for use (Seyoum, 2003). Hence, detail characterization of the coffee germplasm in the country is an important step in identifying coffee varieties with high performance, successful conservation, and utilization of genetic resources for crop improvement. Such knowledge and visualization can be achieved through the study of morphological, structural and functional attributes of germplasm as the carrier of all hereditary characteristics of any given species. Morphological markers in coffee are vital to distinguish variation based on external observation differences, such as size and shape of leaf and plant form, color of the shoot tip, the characteristics of the fruit, angle of branching and the length of internodes (De Vienne *et al.*, 2003).

Starting from 1968, about 6923 germplasm were collected and conserved (JARC Passport data from 1966-2016). Nevertheless, about 15.5% accessions failed to survive in their maintenance field due to climate change and adaptation problem, as they are forced to be grown outside their original environment (Desalegn and Wakuma, 2017). To alleviate such barrier, the conservation program was designed according to their area of origin and specific adaptation to minimize the risk of genetic erosion that may occur due to natural selection (Fikadu *et al.*, 2008). The coffees grown under diverse environments showed

wide genetic variations within and between populations of different regions for yield, quality, disease resistance and other traits (Bayetta *et al.*, 1993). The availability of such genetic variations provides immense possibilities for improvement of the crop, for any desirable traits of interest (Berthaud and Charrier, 1988). Information on crop plant species genetic diversity in their center of origin and of the relationships among elite breeding material has a significant impact on the improvement of crop plants (Hallauer *et al.*, 1988).

Accordingly, genetic variability study has been conducted in coffee geremplasm collected from Sidama, Wollega, Haraghe and Tepi by different researchers. For instance, Olika *et al*. (2011) and Getachew *et al*. (2013) each 49 Limmu coffee accessions and Ermias (2005) on 81 West Wollega coffee accessions, Yigzaw (2005) on 16 North West and South West of Ethiopia coffee accessions, while Mesfin and Bayetta (2005) on Harar coffee accessions at pre-bearing stage have studied the genetic variability of coffee and reported the existence of efficient genetic variability. However, about 58 coffee accessions were collected from Amaro Kele Woreda but not yet characterized for phenotypic and genotypic variability study. Therefore, detailed variability study and information on the extent and nature of interrelationships among characters in Amaro Kele coffee geremlasm is crucial. The current study was undertaken to meet such gap with the following objectives:-

General objective

To assess genetic variability and association of character among *Coffea arabica* accessions collected from Amaro Kele district, Southern Ethiopia using morphological traits.

Specific objective

- To determine level of genetic variability among Amaro coffee collection at Awada using morphological traits
- To determine the level of phenotypic and genotypic association of characters and their direct and indirect relation with coffee yield at Awada, Southern Ethiopia.

2 LITERATURE REVIEW

2.1 Origin and Ecological Requirements of *Coffea arabica*

Arabica coffee has its primary centre of origin and genetic diversity in the high lands of South western Ethiopia (Charrier and Berthaud, 1985; Wrigley, 1988). It is the only species found in Ethiopia (Woldemariam *et al.,* 2002). Its centre of origin is geographically isolated from the centre of origin of other species of the genus *Coffea*. It is confined to the plateau of Southwestern Ethiopia and on the Boma plateau of Sudan (Wellman, 1961; Lashermes *et al.,* 1999; Anthony *et al.,* 2002; Steiger *et al*., 2002). Forests in Southwestern part of Ethiopia are the primary center of origin and center of genetic diversity of *Coffea arabica* (Sylvian, 1958). Ethiopia is the only country in the world, where coffee grows wild as an understorey shrub or small tree in the Afro-montane rainforests (Friis, 1992) viz. Kaffa, Sheka, Yayu Birehane kontire and Anfillo. The wild populations of C. *arabica* are naturally occurring in the undergrowth of the montane rainforest at the altitudes between 1,400 and 1, 900 m a.s.l. (Geber-Egziabher, 1990; Gole *et al.,* 2002). Moreover, Senbeta (2006) observed the highest density of wild coffee at the altitudes between 1,300 and 1,600 m a.s.l, which implies the optimum altitude for wild coffee.

According to Gole (2003) *Coffea arabica* is the afromontane rain forest species of Southwestern and Southeastern part of Ethiopian highlands being grown in diverse environmental factors, such as various altitudes ranging from 1,300 and 1,800 masl. *C. arabica* also grows within the annual rainfall of the country that varies from 1000 to 2400 mm and a wide range of soil types (Soils should be free draining up to a depth of at least 1.5 m and 3 m in drier areas, fertile and acidic to slightly acidic (pH range of 4.5-6) with low availability of phosphorous), where its fertility is maintained by organic recycling (Van der Graaf, 1981). It tolerates annual rainfall between 900 and 1,300 mm yr^{-1}, but most appropriate is above 1,300 mm yr^{-1} with an optimum amount of 1,600 – 1,800 mm yr^{-1}. The temperature requirements for *Coffea arabica* is considered 15-25^{0}C, which prevails in most of the coffee growing areas of the country. The optimum average annual temperature for coffee is 18 – 24 °C with contrasting seasons. The original habitat of coffee is the shaded understory of montane rainforests in Southwestern and Southeastern Ethiopia between 1,000 and 2,000 m asl. Moreover, *C. arabica* grows best in the cool, shady environment of the forest of Ethiopian highlands. Such ecological data are essential for

selecting appropriate growing conditions for living gene banks and for testing of agronomic performance for cultivation (Charrier and Berthaud, 1985).

2.2 Taxonomic Classification and Genetics of Coffee

Coffee-trees belong to the tribe *coffeae* in the large angiosperm family of *Rubiaceae* (Bridson and Verdcourt, 1988), and are classified into two genera: *Coffea* and *Psilanthus*. Charrier and Berthaud (1985) subdivided the genus *Coffea* into two subgenera: *Coffea* (*Eucoffea*) and *Mascarocoffea*. The genus *Coffea* is economically the most important (Wellman, 1961), and comprises more than 124 species (Davis *et al*., 2011). The three species: - *Coffea arabica* L., *C. Liberica* and *Coffea canephora* Pierre, which belongs to the subsection *Erythrocoffea* (Wrigley, 1988) are economically significant (Pearl *et al*., 2004). Out of them, *Coffea arabica* is the most important commercial species and is one of the world's most important commodities (Vega *et al*., 2003; Davis *et al*., 2006).

According to Lashermes *et al*. (1999), *C. eugenioides* and *C. canephora* are the possible ancestor of arabica coffee. There are two types of *C.arabica,* namely: Typica and Bourbon. The "*Typica"* genetic base were transferred from Java to the Amsterdam Botanical Garden and these plants gave rise to the botanical variety of *C. arabica* called "*Typica"* (Wellman 1961). The "Bourbon*"*, genetic base originated from a few coffee trees that were introduced from Mocha (Yemen) to the Bourbon Island (now La Reunion) at about the same time as "*Typica"*.

2.3 Morphology and Reproductive Biology of *Coffea arabica*

Coffee Arabica is an under story shrub or small woody perennial tree that differ greatly in morphology, size and ecological adaptations and it may reach a size of 4 to 5 meters. The plant has a dimorphic habit of branching, in which vertical (orthotropic) branches form horizontal (plagiotropic) branches, which bear the flowers and the fruits in clusters (Van der Vossen, 1974). Flowers of *C. arabica* with short corolla, long style and exerted stamen are typical of the genus Coffea. Such floral morphology would permit natural cross-pollination, but yet, C. arabica is largely autogamous (Lashermes *et al.*, 1996) and fruit set after self-pollination (Carvalho *et al.*, 1969; Van der Vossen, 1974). Most diploid species were proved to be highly self-incompatible, and are allogamous (out crossing). However, in contrast with the widely accepted perception, Meyer (1965) reported the existence of 40% to 60% out crossing rate in wild Arabica coffee populations in Ethiopia. Gezahegn *et*

al. (2014) also confirmed the first formal mating system analysis of *C. arabica* populations based on the inheritance of genetic marker and found an overall multilocus out crossing rate of as high as 76% in its native range.

Inflorescences develop from serial buds, mainly, on horizontal branches. Each inflorescence, normally, carries one to five flowers. The flowers have a short pedicel and a rudimentary calyx. The petals are fused and form corolla with five lobes. The pistil consists of an inferior ovary and a long style with two stigmatic lobes. The ovary is bilocular each with one anatropous ovule (Van der Vossen, 1974). Flower initiation occurs after sufficient rainfall following a dry period. The total period of flowering is normally not more than three days with the majority of flowers opening on the first and the second day (Van der Vossen, 1974). Soon after opening of the flowers early in the morning, the stigma becomes receptive and pollen shedding starts. Withering of flowers occurs in one or two days after pollination. The coffee fruit, usually, contains two seeds. Ripe fruits have a thick fleshy mesocarp and a hard endocarp and each seed is enveloped in a silver skin (testa), which is a remnant of the integument (perisperm) (Van der Vossen, 1974; Urbaneja *et al.,* 1996).

Coffea arabica cultivars are usually propagated by seed, since it is generally believed that *Arabica* coffee is sufficiently true breeding. There is no seed dormancy in coffee because it is recalcitrant type, thus the viability of seeds is short lived and it is advisable to plant the seeds within two to four months after harvesting. This is because older seeds take longer to germinate and could lose viability (Clarke and Macrae, 1988). Vegetative propagation methods are applicable to coffee, including cuttings, grafting and tissue culture. In Ethiopia, vegetative propagation in coffee is done predominantly on the hybrid varieties. Propagation by cuttings is applied when few genotypes need to be propagated in large numbers. *In vitro* methods have also been used for propagation in two ways; micro-cutting or somatic embryogenesis. This multiplication approach is able to produce a great number of plantlets but has the limitation of requiring refined techniques and chemical media (Clarke and Macrae, 1988).

2.4 Production and Contribution of Coffee in Ethiopian Economy

Apart from being the birth place of Coffee *arabica* Ethiopia is also a major producing country of high-value coffee. Global *Coffea arabica* production is about 154.93 million 60

kg bags; while that of African countries is around 16.53 millions of 60kg bags (ICO, 2018). The first six largest global coffee producing countries were Brazil, Vietnam, Colombia, Indonesia, Hondura and Ethiopia (ICO, 2018); while Ethiopia is the most important coffee-producing and exporting country in Africa and the sixth most important coffee producer worldwide (ICO, 2018; USDA, 2018). But, if considering Arabica coffee alone, Ethiopia is the 4th largest producer after Brazil, Colombia and Honduras (ICO, 2018; USDA, 2018). Ethiopia contributes about 4.1 percent of world's Coffee production (USDA, 2018) and 40.7 percent of the total production of coffee in Sub-Saharan Africa (ICO, 2017).

It is estimated that there is a potential six million hectares of cultivable land suitable for coffee production (Mekuria *et al.,* 2004). Ethiopia's annual average coffee production was approximately 4.58 million kg bags over the 2005-2010 periods. Total coffee production has been improving steadily during the past twenty years, with a 110 percent increase between 1993 and 2011. However, the volume of coffee produced decrease, although the level of area cultivated continued to increase due to lack of improved variety. Coffee is mainly grown by smallholder farmers on less than 1 hectare of land, and earning less than a dollar per day (McCarthy, 2007).

Coffee cultivation plays a vital role both in the cultural and socio-economic life of the nation. About 35% of the total production is consumed within the producing areas and in general, about 45% of the coffee produced is consumed within Ethiopia (ICO, 2017). The crop is mainly, produced in the Southern, South western and Eastern parts of the country. The total area coverage of coffee in Ethiopia is estimated to be around 700,474.69 ha, of which about 95% is produced by 4 million small scale farmers (Arslan and Reicher, 2011; CSA, 2017), whereas the estimated annual national production of coffee is about 7.00 million 60kg bags (CSA, 2015) and 7.83 million 60kg bags (CSA, 2017)**.** The average national production was about 670 kg ha^{-1} (CSA, 2017). The Southern region is the second region next to Oromia regional state in coffee producing areas and production. According to CSA, (2017), in the Southern region about 217,307.11 ha of land was cultivated and 148.34 million kg was produced with average yield of 683kg ha^{-1} (CSA, 2017) and in the Segen People's Zone of SNNPS from 1,181.72 ha of land, about 1,003,252 kg was produced with average yield of 849 kg ha^{-1}.

The four major types of commercial coffee are Jimma, Sidama, Yirgacheffe, and Harar. The names correspond to the cities around which they are produced. These four coffee trademarks represent around 70 % of the total coffee exports registered by the Ethiopian Revenue and Customs Authority from 2004 to 2009 (Arslan and Reicher, 2011). Ethiopia's main trading partners from 1995 to 2011 were Germany (29 %), Saudi Arabica (15 %) and Japan (16 %). However, Japan has strongly reduced imports, since 2007, after traces of pesticides were discovered on packing bags. Export to the United States is also on the rise, their share of total exports increasing from 4 % to 8 % between 2000 and 2011 (FAO, 2014). The percent share of Ethiopian coffee to the world coffee market in the four consecutive years has been 4.1, 4.1, 4.4 and 4.3 in 2013, 2014, 2015 and 2016 year of production, respectively (CSA, 2017).

2.5 Genetic Diversity of *Coffea arabica* in Ethiopia

Variability is the occurrence of differences among individuals due to differences in their genetic composition and/or the environment in which they are raised (Allard, 1960). If the character expression of two individuals could be measured in different environment identical for both environments, differences in expression would result from genetic control, and hence, such variation is called genetic variation (Falconer and Mackay, 1996). Evaluation of genetic diversity within a crop plant is important, as it determines the extent to which the crop can be improved or changed through selection. Information on the nature and magnitude of genetic variability present in a crop species is important for developing effective crop improvement program (Dabholkar, 1999).

Genetic variability, which is due to the genetic differences among individuals within a population, is the core of plant breeding because proper management of diversity can produce permanent gain in the performance of plant and can buffer against seasonal fluctuations (Sharma, 1998). These genetic variations can be enumerated at three levels: species, populations and individual levels. Since Ethiopia is the only centers of origin and diversifications of *Coffea arabica,* there is a high genetic diversity, which is mainly attributed to its diverse ecological features, such as suitable altitude, ample rainfall, optimum temperature, fertile soils and the presence of indigenous methods of coffee production system in the country (Gole *et al*., 2001; Yeshitila *et al.*, 2004).

Higher genetic diversity of C. arabica exist in Ethiopia than anywhere else in the world, which has led botanists and scientists to consent that Ethiopia is the center of origin, diversification and dissemination of the coffee plant (Mekuria *et al.,* 2004). Several phenotypic and molecular studies revealed that the populations of *C. arabica* from the Southwestern part of Ethiopia have high genetic variability, and the forests there are, thus, suitable for *in situ* conservation of the species. For example, Sylvian (1955, 1958) and Meyer (1968) observed high diversity of several phenotypic characters among the Ethiopian coffee populations. Montagnon and Bouharmont (1996) also found higher phenotypic diversity among the populations of *C. arabica* collected from Ethiopia, as compared to cultivated populations of the species from around the world. According to the study of genetic variation among forty nine *Coffea arabica* accessions from Limu, Ethiopia confirmed the presence of trait diversity within coffee accessions (Olika *et al*., 2011). The study of genetic variation among 100 *Coffea arabica* accessions from Hararghe, Ethiopia were also confirmed the presence of trait diversity within 14 characters suggesting that the presence of high variability among the accessions (Kebede and Bellachew 2004; 2008). In general, the presences of significant difference between Arabica coffee accessions for different characters were reported (Walyaro and Van der Vossen, 1979; Walyaro, 1983; Marandu *et al*., 2004; Mesfin and Bayetta, 2005; Yigzaw, 2005; Getachew *et al*., 2013; Atinafu and Mohammed, 2017 and Beksisa *et al.,* 2017).

2.6 Genetic Diversity Assessment Methods

Like it is for many crops, evaluation of the genetic diversity and available resources within the genus Coffea is an important step in coffee breeding (Cubry *et al.,* 2007). As new coffee varieties are continuously being developed through hybridization, there is a need to determine the level and sources of genetic variation within and between new and existing coffee varieties (Gichimu and Omondi, 2010). Genetic variation of coffee can be assessed using different techniques like: - Morphological, biochemical, molecular and physiological markers are the approaches used to estimate genetic diversity among crop species (Mehmood *et al.,* 2008). Morphological evaluation is recognized as the most widely used (Geleta and Labuschagne 2005), as it makes it possible to dissect differences among genotypes (Bucheyeki *et al.,* 2009) with less cumbersome and sophisticated techniques, and more importantly, under the actual crop performance conditions.

2.6.1 Morphological assessment

Morphological characteristics were among the earliest genetic markers used for the assessment of variation and are still of great importance. Usually, these characters are inexpensive and simple to score. The sharing of physical features is also often accepted as an indication of relatedness. There are several sets of physical character assessment for different crops at different developmental stages, such as seed, juvenile, adult vegetative, flower and fruit. However, these sets of characters lack adequate coverage of the genome, strongly influenced by environmental factors, might be controlled by several genes. Besides, assessment of morphological characters in perennial plants, such as coffee, often requires a lengthy and expensive evaluation, during the whole vegetative growth. Morphological markers are cheap and easy to apply; sensitive to environmental influences and developmental stage of the plant. Different scholars reported that although agro-morphological characters are often influenced by environmental conditions, the method is still useful and easy to apply for classification, estimating diversity and registration of cultivars. The entire diversity of C. *arabica* is confined in the afromontane rainforest located in the west and east of the Great Rift Valley (Essayas, 2005). Study on the morphological characters on *C. arabica* in Ethiopia has confirmed the presence of high phenotypic diversity among germplasm collected and maintained in the *ex situ* gene bank of Ethiopia (Kebede and Bellachew, 2004; Seifu *et al.*, 2004). Moreover, the analysis made on the materials outside Ethiopia illustrated the existence of variation that were grouped as east-west of the Great Rift Valley (Montagnon and Bouhannont, 1996).

Sylvain (1955) classified Ethiopian cultivated coffee into 13 main types namely, *Agaro, Arbagugu, Cioiccie, Coulo, Dilla, Ennarea, Harar, Yirgalem, Keffa, Tafari Kela, Wolkite, Wollamo* and *Zeghie* using only bean and fruit morphology. Montagnon and Bouharmont (1996) classified wild and cultivated coffee genotypes from Ethiopia based on their geographic origin using 18 agro morphological traits. The FAO coffee collection team has observed phenotypic variation in branching habit, young leaf color, fruit color, persistence of sepals, leaf and fruit size. Mesfin (1986) observed growth habit variation, such as compact and spreading type genotypes from national coffee collections. Likewise, Selvakumar and Sreenivasan (1989) observed phenotypic variation among 54 coffee accessions collected from Keffa province of Ethiopia. In coffee, it is important to identify the most suitable age of a tree when characters can be measured easily and with utmost accuracy because coffee is a perennial crop. Walyaro (1983) reported that characters such

as tree height, girth of the stem, internodes length measured on the main stem and primaries and radius of canopy can be accurately determined using a single measurement even on young trees, 18 months after field planting. Genetic studies in arabica coffee have shown that selection efficiency for higher bean yield can be increased by taking into account various growth parameters and yield components, such as stem girth, canopy radius, percentage of bearing primaries, percentage of bearing nodes and number of berries per node (Van der Vossen, 1985).

2.6.2 Molecular marker

Molecular markers have been replacing or complementing traditional morphological and agronomic characterization, since they are virtually unlimited, cover the whole genome, are not influenced by the environment, and less time consuming. Each molecular marker has its advantages and drawbacks. Application of molecular marker techniques to diversity questions must take into account, whether or not the data derived from a technique provide the right type of information for answering the question being addressed (Karp *et al.,* 1997). The choice of appropriate molecular markers depends on the accessibility and cost effectiveness of the marker techniques. Research were done on forest coffee populations in Ethiopia to determine the extent and distribution of its genetic diversity using PCR based DNA marker techniques such as random amplified polymorphic DNA (RAPD), inverse sequence-tagged repeat (ISTR), inter-simple sequence repeats (ISSR) and simple sequence repeat (SSR) or microsatellites (Powell *et al.,* 1996).

In coffee, DNA-based molecular marker technology has already been implemented in germplasm characterization and management, detecting genetically divergent breeding subpopulations (for example to predict hybrid vigour), establishing gene introgression from related species and molecular marker-assisted selection (Lashermes *et al.,* 1996). However, the molecular phylogeny of *Coffea* species has been established using DNA sequence data. The molecular markers have revealed an extremely reduced genetic diversity in *Coffea arabica* L. in comparison to *C. canephora* (Etienne *et al.,* 2002). Anthony *et al.* (2001, 2002) reported the presence of high genetic diversity based on RAPD (Random Amplified Polymorphic DNA) markers, AFLP (Amplified Fragment Length Polymorphism) and SSRs (Simple-Sequence Repeats) markers. Material originating from Ethiopia and the arabica sub-groups *C. arabica* var. typica and *C. arabica* var. bourbon were clearly distinguished. Orozco-Castillo *et al.* (1994)

demonstrates the power of the polymerase chain reaction technology for the generation of genetic markers for long-lived perennial tree and bush crops.

Pearl *et al.* (2004) used AFLPs to construct a genetic linkage map on a pseudo-F2 population of arabica coffee (*Coffea arabica* L.) derived from a cross between the cultivars Mokka hybrid and Catimor. The recent analysis of the genetic diversity among forest populations in Ethiopia with different marker systems also showed moderate to high polymorphisms and groupings resulted based on geographic origin (Essayas, 2005). Dessalegn *et al*. (2009) indicated that AFLP markers were more efficient compared to SSR markers for characterization of the evaluated coffee genotypes. Molecular markers provide the best estimate of genetic diversity since they are independent of the confounding effects of environmental factors (Powell *et al.,* 1995). The use of molecular markers of the SSR and ISSR types in the study of diversity was efficient in carrying out the molecular characterization of coffee genotypes between and within C. arabica and C. canephora. Motta *et al.* (2014) reported that microsatellites markers were efficient in estimating the genetic similarity and could be used to increase the efficiency in classifying the materials.

2.6.3 Biochemical markers

Enzymes are the basic tools of cellular chemistry and were introduced as markers in the early 1970s (Glaubitz and Moran, 2000). Isozymes were the first molecular markers used in plant breeding. A number of studies conducted in the early 1950s provided evidence regarding the existence of multiple forms of enzymes (McMillin, 1983). Isozymes revealed when tissue extracts are subjected to electrophoresis in various types of gels and subsequently submersed in solutions containing enzyme-specific stains. Isozyme studies in plants have demonstrated that pattern and band intensities differ by tissue types and developmental stages (Montarroyos *et al.,* 2003). Although isozymes are not as plentiful as DNA markers and limited by tissue and developmental stage specificity, it has been used for genetic diversity analysis in many species (Dudnikov, 2003). The isozyme technique appears to be more informative at lower taxonomic levels, particularly for species and population level characterization (Brown, 1990). Isozymes have been applied to C. arabica. However, their use for arabica coffee characterization have been limited due to the small number of isozyme systems available (Berthaud and Charrier, 1988).

2.7 Phenotypic and Genotypic Coefficients of Variation

Naturally occurring genetic variability is useful in any plant breeding program. It is the amount of the total genotypic and phenotypic variability that exists in a crop germplasm that dictates the initiation of crop improvement programs and develops better varieties. Of the total variability present in a population the genetic component is most important to the breeder, as it could be transmitted to the progeny. In addition, proper management of this type of variability can produce permanent gain in the performance of the crop concerned (Mayo, 1980 and Welsh, 1990). Phenotypic variability is the observable traits of variation present in a population; and it is a combined effect of genotypic value and environmental deviation. Genotypic variations, on the other hand, is the component of variation, which is due to the genetic differences among individuals within a population, and is the main concern of plant breeding (Singh, 2001).

In Ethiopia the geographic allocation of coffee within its homeland is good indication for the existence of genetic variation within a population. Variability in coffee Arabica has been reported to exist in different locality, where the crop is grown. Different cultivars have been distinguished on the basis of morphological (plant height, branching habit, leaf colour, leaf shape internodes length bean size and stem girth). Wide range of variability with respect to these characters has been observed for different accessions. Such traits of variability could enable Ethiopian coffee breeders to screen for coffee berry diseases resistant varieties and heterotic hybrid cultivars through crossing. Yigzaw (2005) reported that the estimates of PCV and GCV in coffee accessions for 18 quantitative characters ranged from PCV and GCV ranged from 4.5 to 53.4% and 3.3 to 51.7%, respectively. Getachew (2012) also reported high PCV (91.5 and 41.7%) and GCV (62.8 and 22.1%) values for CBD reaction and yield per tree, respectively. Similarly, Olika *et al*. (2011a) and Getachew (2012) have reported high PCV and GCV values for coffee berry disease reaction and yield per tree; moderate PCV and GCV values for height up to first primary branch and hundred bean weights.

2.8 Heritability and Genetic Advance

Information on the nature and magnitude of variability and heritability in a population is one of the prerequisites for successful breeding program in selecting genotypes with desirable characters (Dudly and Moll, 1969). It is, therefore, of great importance for breeders to know the heritability of the agronomical characters to improve the yield of the

crop effectively. According to Falconer and Mackay (1996), heritability is defined as the measure of correspondence between breeding values and phenotypic values. Thus, heritability plays a predictive role in breeding, and expressing the reliability of phenotype, as a guide to its breeding value. It is the breeding value which determines how much of the phenotype would be inherited in to the next generation (Tazeen *et al.,* 2009).

Heritability can be either broad sense or narrow sense. The broad sense heritability is the relative magnitude of genotypic and phenotypic variance for the traits and it gives an idea of the total variation accounted to genotypic effect (Allard, 1960). This gives an idea of the total variation ascribable to genotypic effects, which are exploitable portion of variation. Heritability in the narrow-sense is important as it affects genetic gain that depends on the proportion of additive genetic variance to phenotypic variance (Falconer and Mackay, 1996). It can also be used to establish the proper weighting of information from different types and numbers of relatives to achieve the best estimate of breeding value.

There is a direct relationship between heritability and response to selection, which is referred to as genetic advance. High genetic advance with high heritability estimates offer the most effective condition for selection (Larik *et al.,* 2000). The utility of heritability therefore increases when it is used to calculate genetic advance, which indicates the degree of gain in a character obtained under a particular selection pressure. Breeding most effective yield component, through yield improvement can be achieved, if the component traits are highly heritable and positively correlated with yield**.** However, it is very difficult to assess whether observed variability is highly heritable or not, due to polygenic nature of quantitative traits. Likewise, knowledge of heritability is essential for selection based improvement, as it indicates the extent of transmissibility of a character into future generations (Sabesan *et al*., 2009, Ullah *et al*., 2011).

Genetic advance expected from selection refers to the improvement of characters in genotypic value for the new population compared with the base population under one cycle of selection at a given selection intensity (Singh, 2001). Since high heritability does not always indicate high genetic gain, considering both heritability and genetic advance need to be used in predicting the ultimate effect for selecting superior varieties (Ali *et al*., 2002). Genetic advance gives clear picture and precise view of segregating generations for possible selection. Higher estimates of heritability coupled with better genetic advance

confirms the scope of selection in developing new genotypes with desirable characteristics (Ajmal *et al.*, 2009).

According to Atinafu *et al.* (2017) the estimate of the broad sense heritability for coffee leaf rust reaction and for leaf length is 50.47 and 90.30, respectively. The recorded estimates of heritability are high (>50%) for leaf length, average internode length of main stem, stem diameter, coffee berry disease reaction, canopy diameter, number of main stem nodes, average length of primary branches, plant height, number of primary branches, percent of bearing primary branches, angle of primary branches, fruit length, hundred green bean weight, bean weight, average green bean yield, leaf width, leaf area and coffee leaf rust reaction (Yigzaw 2005; Olika *et al.,* 2011a and Atinafu *et al.,* 2017). However, Getachew (2012) indicated the moderately low heritability for fruit length (48.08%), coffee berry disease severity (47.15%), plant height (47.05%), average internode of main stem (41.38%), leaf length (41.28%), number of primary branches (39.40%),average length of primary branches (36.91%) and clean coffee yield per tree (28.00%). Ermias (2005) has observed low heritability for percent bearing primary branches (13%).

The estimates of genetic advance as percent of mean (GAM) that could be expected from selecting the top 5% of the coffee genotypes were high for CBD (108.08%), CLR (45.07%), average green bean yield (35.62%), stem diameter (29.82%), average internode length of stem (26.02%), number of primary branches (25.60%), plant height (24.45%) and average length of primary branches (23.46%) (Atinafu *et al.,* 2017). Similarly, Abdi (2009) reported that high GAM for green bean yield per plant (111.4), leaf area (56.4), number of secondary branches (35.0), leaf width (34.7), leaf length (27.9) and 100 green bean weights (23.8%). This author also reported moderate GAM for number of main stem nodes (19.92%), number of secondary branches (19.52%), hundred green bean weight (19.14%), leaf length (17.58%), angle of primary branches (17.54%), percent bearing primary branches (16.94%), canopy diameter (16.17%), leaf area (14.63%) and leaf width (12.34%) and low GMA for fruit length (7.06%) and bean width (6.62%). In addition, Yigzaw (2005) observed relatively high values of genotypic coefficient of variation, broad sense heritability and genetic advance for various characters.

Furthermore, the combined use of genetic coefficient of variation, heritability and genetic advance seems vital for effective improvement of a particular trait in a population (Yigzaw, 2005). The high estimates of heritability, coupled with, high genetic advance as

percent of means were observed for characters, such as coffee berry disease, coffee leaf rust, average green bean yield, stem diameter, average internode length of main stem, plant height, number of primary branches and average length of primary branches, which might show the importance of additive gene effects and improvement through selection based on phenotypic performance can be effective (Yigzaw, 2005). Similar, results in coffee were also reported by several researchers (Ermias, 2005; Yigzaw, 2005; Abdi, 2009; Olika *et al.,* 2011a; Getachew, 2012). According to Olika *et al.* (2011a), the expected genetic advance as percent of the mean from selecting the top 5% of the genotypes varied between 0.11 to 77.64% for height up to first primary branches, number of secondary branches, hundred green coffee bean weight and yield of clean green coffee per tree in arabica coffee accessions showed higher heritability and genetic advance.

2.9 Correlation Studies

Creative crop improvement scheme refers to the collection of superior alleles into single targeted genotype (Tripathi *et al.,* 2011). The nature and extent of genetic variation governing the inheritance of characters and association will facilitate effective genetic improvement. It is noticeable that information of morphological and physiological aspects of crop is also a key feature to plan a resourceful breeding program. Thus, the genetic reconstruction of plant architecture is required for developing high yielding crop varieties (Yadav *et al*., 2011).

It is imperative that breeders need to understand the magnitude of variation, correlation and inheritance of important agronomic traits. Yield in perennial crop is one of the most important and complex traits in plant breeding experiments. Continued improvement of yield remains the top priority in most of the breeding programs. In coffee, the outcome of yield depends on various growth characters, and their combinations, such as stem girth, canopy width, number of primary branches and number of secondary branches (Dancer, 1964 and Srinivasan, 1982). In addition, a number of other agronomic characters; such as plant height, leaf area, number of nodes on primary branches and number of fruits can directly or indirectly influence yield (Mesfin ,1982). Hence it is important understand the relationship between yield and other agronomic characters to improve yield and yield related traits, because it is influenced by all factors that determine productivity (Araus *et al.,* 2001). It is, therefore, valued to estimate the magnitudes of associations among the yield and yield component traits.

Correlation coefficient quantifies the relationship between two variables. It simply measures mutual association without cause and effect relationship (Dewey and Lu, 1959). Correlation analysis is a handy technique, which provides information that selection for one character results in progress for other positively correlated characters (Manggoel *et al.*, 2012). The importance of correlation studies in selection program is appreciable, when highly heritable characters are associated with the traits of interest, like yield. Correlation coefficients, although very useful in quantifying the size and direction of trait associations, can be ambiguous, if the high correlation between two traits is a consequence of the indirect effect of other traits (Bizeti *et al*., 2004).

A positive value of correlation shows that the changes of two variables are in the same direction, specifically high value of one variable are associated with high values of the other and vice versa. When correlation is negative the movements are in opposite directions, that is, high values of one variable are associated with low values of the other (Yadav *et al*., 2011). Depending on the sign of genetic correlations between two traits can either facilitate or impede selection progress. Correlation value (r = 1) implies perfect (100%) correlation, where both traits vary hand in hand, while (r = -1) means, there is 100 % correlation between two characters, but they vary in opposite direction, and (r = 0) carries the implication that there is no correlation at all between the two characters (Falconer and Mackay, 1996).

Correlation can be measured in different indices (coefficient) based on different statistical hypothesis, i.e., Pearson correlation coefficient, Spearman rank correlation coefficient and Spearman semi-quantitative correlation coefficient, Gamma correlation coefficient (Rosner ,1995). For example Karl Pearson (1857 - 1936) coined the Pearson product-moment correlation coefficient ($\mathbf{r_{prs}}$= Pearson correlation coefficient) and a major contributor to the early development of statistics. Assumes both variable (variables X and Y) are interval or ratio variables and are well approximated by a normal distribution, and their joint distribution is bivariate and normal. Pearson correlation coefficient can take values from -1 to +1 and considering strong correlation, if the correlation coefficient is greater than 0.8 and a weak correlation, if the correlation coefficient is less than 0.5 (Spearman, 1904). Several correlation studies indicated that the quantitative characters like number of stem nodes, primary branches, plant height, length of the longest primary branch and stem diameter have positive and negative correlation with yield, and such traits

could be used as a selection criterion for improving the productivity of the crop, since they represent the lion's share in the variability of the coffee population in the specified area (Gessese *et al*., 2015).

The presences of significant difference between Arabica Coffee accessions for different characters were reported by Walyaro and Van der Vossen (1979); Walyaro (1983); Marandu *et al*. (2004); Mesfin and Bayetta (2005); Yigzaw (2005); Olika *et al*. (2011a); Getachew *et al*. (2013); Atnafu and Mohammed (2017). Coffee yield had positive genotypic and phenotypic correlations coefficients with all characters except height up to first primary branch. Among the characters studied, the correlation was statistically significant with number of primary branch, canopy diameter, number of main stem nodes and main stem diameter, indicating greater importance and reliability of these characters for improvement of yield in coffee. As one of these characters is improved, an enhancement or improvement of coffee yield is also achieved (Beksisa *et al.,* 2017). Yigzaw (2005) and Olika *et al*. (2011a) reported positive and significant correlation for most of the quantitative characters with yield. Srinivasan (1980) reported high positive correlation of stem girth and length of primary branches with yield. Similarly, Walyaro and Van der Vossen (1979) also reported significant and positive genotypic correlations between yield and girth at the base of the main stem. Walyaro (1983) and Marandu *et al*. (2004) also reported that coffee yield is influenced by most important characters, like number of primary branches, canopy diameter, plant height and main stem diameter.

Similarly, Ermias (2005) also reported weak and non-significant correlation of internode length with average yield. In this study, yield was significantly and negatively correlated with only height up to first primary branch for both genotypic and phenotypic levels. In addition, canopy diameter, plant height and main stem diameter showed significant and positive correlation with most of the characters (Olika *et al*., 2011a and Beksisa *et al.*, 2017). In studies of genetic divergence and the process of evaluation and selection, it is important to maintain traits that are correlated with the majority of traits (Ferrao *et al*., 2008). Similar results were reported by Marandu *et al*. (2004) in Robusta coffee. Likewise, correlation of canopy diameter with internode length, number of main stems node, plant height, main stem diameter and yield was positive and significant.

2.10 Path Coefficient Analysis

Path coefficient analysis is a very important statistical tool that indicates which variables (causes) exert influence on other variables (responses), while recognizing the impacts of multi-co-linearity (Akanda and Mundt, 1996). Path coefficient analysis can be defined as "the ratio of standard deviation of the total effect" (Falconer and Mackay, 1996). Path coefficient analysis is a measure of the direct and indirect effects of each character on bean yield, estimated using a standardized partial regression coefficient known as path coefficient analysis, as suggested by Dewey and Lu (1959).

rij =Pij+ Σrikpkj Where:- rij = Mutual association between the independent character (i) and dependent Character (j) as measured by the correlation coefficient ,Pij = Component of direct effects of the independent character (i) on dependent character, (j)as measured by the path coefficient and, ∑Rikpkj = Summation of components of indirect effect of a given independent character (i) on the given dependent character (j) via all other independent character (k). Residual effect will be estimated by the formula:

,Where: - R^2 =Σpijrij pij = Component of direct effects of the independent character (i) and dependent character (j) as measured by the path coefficient.rij = Mutual association between the independent character (i) and dependent character (j) as Measured by the correlation coefficient.

The variable yield is the result of interaction between component traits, which are either positively or negatively associated with each other. The path coefficient analysis furnishing the cause and effect of different yield components would provide better index for selection, rather than, mere correlation coefficients. Path coefficient analysis partitions the genetic correlation between yield and its component traits into direct and indirect effects, and hence, has effectively been used in identifying useful traits, as selection criteria to improve yield (Akinwale *et al*., 2011; Sadeghi, 2011). Yield in coffee is commercially an important trait, which is considered in most, if not all, breeding goals of coffee improvement. Therefore, it is desirable to know the direct and indirect effects of yield related traits in coffee. These traits could be useful indicators in breeding programs to select coffee genotypes for yield.

The main selection criterion in coffee is yield, quality and disease resistance. Other agronomic characters related to yield potential have been studied to increase the indirect

selection efficiency. Beksia *et al.* (2017) reported that positive direct effect on coffee yield exerted by plant height (1.564), canopy diameter (1.555**)** and length of first primary branch (0.052), which indicates that these traits are effective for the improvement of coffee yield. Internodes length (-1.860) and number of primary branches (-1.802) also exerted high and negative effects on yield. In the contrary, length of first primary branch (0.052), followed by number of main stem nodes (-0.232) showed the lowest direct effects on yield. However, the encountered indirect effect of number of main stem nodes via plant height (1.068) was relatively high. Ermias (2005) also observed positive direct effect of plant height, but negative direct effects of canopy diameter and length of primary branch on yield. Moreover, Srinivasan (1980) reported that greater weight should be given for longer primaries and shorter internode in selection for yield, as they had direct positive effects.

On the other hand, internodes length (-1.860), number of primary branch (-1.802), height up to first primary branch (-0.609), main stem diameter (-0.444) and number of main stem nodes (-0.232) which had positive genotypic and phenotypic correlation coefficient with yield except height up to first primary branch exerted negative effect on yield (Beksisa *et al.*, 2017). Similarly, internodes length revealed positive indirect effect on yield through almost all characters, except height up to first primary branch and main stem diameter. Number of main stem node also indirectly exerted positive effects on yield via all characters except number of primary branch, length of first primary branch and main stem diameter. Main stem diameter indirectly exerted positive effects via canopy diameter, length of first primary branch and plant height.

2.11 Divergence Analysis (D^2)

Genetic diversity refers to the variation among alleles of genes in different individuals of population of a species (IPGRI, 1993). Van Hintum (1995) indicated that genetic diversity studies based on genetic markers and qualitative characters are used for many purposes which includes: - for taxonomic studies, to find the center of diversity of a species, to trace the route of domestication, to study the relationship between environment and diversity and to study a complete crop gene pool or the diversity of intra-specific part of a gene pool. Divergence analysis used to estimate the genetic distance/divergence of the coffee geremplasm populations or might be used to classify the divergent genotypes into different groups. It also measures the forces of

differentiation at intra (Genotypes grouped into the same cluster presumably, diverge little from one another, as the aggregate characters are measured) - and inter-cluster levels, and determines the relative contribution of each component trait to the total divergent (Mohammadi and Prasanna, 2003).

2.12 Principal Component Analysis

Principal component analysis (PCA) is the most frequently used multivariate method (Crossa, 1990; Purchase, 1997). Its aim is to transform the data from one set of coordinate axes to another, which preserves, as much as possible, the original configuration of the set of points and concentrates of the data structure in the first principal component axis. Various limitations have been noted for this technique (Zobel *et al*., 1988). Crossa (1990) pointed out that the linear regression method uses only one stastics i.e., the regression coefficient, to describe the pattern of response of a genotype across environments, and most of the information is wasted in accounting for deviation. Principal component analysis (PCA) is a generalization of linear regression that overcomes this difficulty by giving more than one stastics i.e., the scores on the principal component axes, to describe the response of a genotype. Masreshaw (2018) reported that traits such as:- average inter-node length of primary branches, average length of primary branches, canopy diameter, fruit width, fruit thickness, bean width, bean thickness and hundred bean weight contributed more to the total variation. Yigzaw (2005) also reported characters contributing for variation among coffee genotypes like inter-node lengths, tree height, canopy diameter, number of branches, bean and fruit characters.

2.13 Cluster Analysis

Cluster analysis is a numerical classification technique that defines groups of clusters of individuals. The first is non-hierarchical classification, which assigns each item to a class. The second type is hierarchical classification, which groups the individuals into clusters and arranges these into a hierarchy for the purpose of studying relationships in the data (Crossa, 1990). Moreover, cluster analysis is multivariate method that groups observations into clusters. Its objective is to sort genotypes into groups, or clusters, so that degree of association will be strong between members of the different cluster and weak between members of same clusters.

Hierarchical cluster methods produce a hierarchy of clusters from small clusters of very similar items to large clusters that include more dissimilar items. A hierarchical method usually, produces a graphical output known as a dendrogram or tree that demonstrates this hierarchical clustering structure. Some hierarchical methods are divisive; those progressively divide the one large cluster comprising all of the data into two smaller clusters and repeat this process until all clusters have been divided. Other hierarchical methods are agglomerative (round mass collection) and work in the opposite direction by first finding the clusters of the most similar items and progressively adding less similar items until all items have been included into a single large cluster (Mohammadi and Prasanna, 2003). Multivariate analysis of morphological quantitative characters and qualitative characters (using cluster analysis) has been used previously to measure genetic relationships within crop, species; examples include coffee (*Coffee Arabica L*.) (Olika *et al*., 2011b; Getachew *et al*., 2013).

The phenotypic similarity of 124 coffee genotypes was assessed by cluster analysis using 19 quantitative characters (Atinafu *et al.,* 2017). Cluster analysis confirmed the presence of some variation among genotypes. The 124 coffee genotypes were grouped into ten clusters. The majority of accessions (114 or 91.93%) were classified in to four clusters (47, 30, 23 and 14 genotypes) in clusters I, II, III and IV, respectively. Others clusters had from 1 up to 2 members. Each clusters V, VI, VII and VIII had two accessions (1.61%) of the total population and clusters VX and X had one accession (0.08%) for each in the total population, indicating that coffee accessions of the same cluster group were at least morphologically similar (Atinafu *et al.,* 2017). Abdi (2009) reported phenotypic diversity among 49 Harerge coffee accessions for 16 quantitative characters were grouped into 6 clusters. Similarly, Olika *et al.* (2011b) has made cluster analysis based on 22 quantitative traits that grouped 49 Limmu coffee genotypes in to four clusters.

However, Atinafu *et al.* (2017) clustered 124 coffee accessions into 10 distinct groups based on seven qualitative traits. Cluster-IV was the largest and consisted of 32 accessions (25.81%) followed by cluster-I (16.94%), cluster-II (13.71%), cluster VI (12.90%), each cluster-III and cluster V (11.29%), cluster VIII (3.23%), cluster VII (2.43%) and cluster XI (0.81%). Accessions grouped under cluster VI have predominately intermediate growth habit, strong stem, and many primary branches with many secondary branches, lanceolate leaf shape, brownish-tipped young leaves, obovate fruit shape and light red fruit color.

Cluster XI, on the other hand, comprised of two accessions. Similarly, the 19 coffee accessions were classified into five distinct groups (Tounekti *et al.*, 2017). Cluster II had the highest number of accessions with eight accessions (44% of the total population), followed by cluster I with four accessions (22%). Clusters III had three accessions (17%), while clusters IV and V had two (11%) and one (6%) accessions, respectively (Tounekti *et al.*, 2017).

3 MATERIALS AND METHODS

3.1 Description of Experimental Site

The experiment was carried out at Awada Agricultural Research Sub-Center that was established in 1997 on land area of 31 ha near Yirgalem town, 45 km south of Hawassa and 319 km from Addis Ababa. Awada is located at 06˚44′ 57'' N latitude and 038˚23'16''E longitude and at an altitude of 1738 meters above sea level (m.a.s.l). The mean annual rainfall of the area is 1342 mm with an average maximum and minimum air temperatures of 28.4 ^{o}C and 11.^{o}C, respectively. The major soil types of the center are eutric-nitosol and chromotic-cambisols that are highly suitable for coffee production (Mesfin and Bayetta, 2008).

3.2 Planting Materials

About 58 coffee germplasm accessions were collected from ten representative peasant associations of Amaro woreda of Segen people zone, see (Fig.1) below. Since, the previous conventional approach largely focused on the development of widely adapted varieties, improved local varieties for each specific agro-ecology are lacking in most of the coffee growing areas. Therefore, collection was made in 2013 to address this locality through coffee genetic resources collection for further coffee improvement program that might help to develop coffee varieties that have paramount importance to promote and maintain the existing speciality coffee quality heritage of the area. Hence, 64 coffee accessions including six pure line checks (Angafa, Feyate, Koti, Odicha, 74112 and 7440) were used for the study. Accessions were established under uniform *Sesbania sesban* temporary shade trees and the other management practices like: - pruning and slashing were also uniformly applied as per the coffee agronomic production practices.

Table 1 Geographical origin of the studied coffee (Coffea arabica L.) germplasm accessions used in the study

Acc.No	Kebele	S/Location	Alt (m asl)	Acc.No	Kebele	S/Location	Alt (m asl)
Ak-1	Kereda	Hurbo	1380	Ak-30	Danobulto	Shashe	1500
Ak-2	Kereda	Hurbo	1380	Ak-31	Kele kebele	Tsele	1900
Ak-3	Kereda	Hurbo	1380	Ak-32	Kele kebele	Tsele	1900
Ak-4	Kereda	Hurbo	1380	Ak-33	Kabo	Tsele	2000
Ak-5	Kereda	Hurbo	1380	Ak-34	Kabo	Tsele	2000
Ak-6	Kereda	Hurbo	1380	Ak-35	Kele	Tsele	1900

Acc.No	Kebele	S/Location	Alt (m asl)	Acc.No	Kebele	S/Location	Alt (m asl)
Ak-7	Golbe	Gudeda	1880	Ak-36	Kele town	Kele-01	1600
Ak-8	Golbe	Golbe	1880	Ak-37	Kele town	Kele-02	1600
Ak-9	Golbe	Golbe	1880	Ak-38	Kele town	Kele-03	1600
Ak-10	Golbe	Golbe	1880	Ak-39	Kele town	Kele-04	1600
Ak-11	Golbe	Golbe	1880	Ak-40	Sharo	Angushi sharo	1600
Ak-12	Golbe	Golbe	1880	Ak-41	Sharo	Angushi sharo	1600
Ak-13	Golbe	Gudeda	1880	Ak-42	Sharo	Angushi sharo	1600
Ak-14	Kerma	Dogodo-1	1660	Ak-43	Sharo	Angushi sharo	1600
Ak-15	Kerma	Dogodo-1	1660	Ak-44	Sharo	Angushi sharo	1600
Ak-16	Kerma	Dogodo-2	1660	Ak-45	Sharo	Angushi sharo	1650
Ak-17	Kerma	Dogodo-3	1660	Ak-46	Sharo	Angushi sharo	1650
Ak-18	Kerma	Dogodo-4	1660	Ak 47	Darba	mane na	1700
Ak-19	Kerma	Dogodo-5	1580	Ak 48	Darba	Sibale	1670
Ak-20	Kerma	Dogodo-6	1580	Ak 49	Darba	Sibale	1670
Ak-21	Kerma	Merere-1	1580	Ak 50	Darba	mane na	1690
Ak-22	Kerma	Merere-1	1580	Ak 51	Darba	mane na	1590
Ak-23	Danobulto	Shashe	1500	Ak 52	Tifata	Tsilalo omo	1680
Ak-24	Danobulto	Shashe	1500	Ak 53	Tifata	Tsilalo omo	1680
Ak-25	Danobulto	Shashe	1500	Ak 54	Tifata	Kepe	1650
Ak-26	Danobulto	Shashe	1600	Ak 55	Tifata	Kepe	1650
Ak-27	Danobulto	Shashe	1600	Ak 56	Tifata	Afa Tsilalo	1700
Ak-28	Danobulto	Shashe	1600	Ak 57	Tifata	Abetu kotsare	1700
Ak-29	Danobulto	Shashe	1500	Ak 58	Gumute	Boyo	1600

3.3 Experimental Design and Field Management

The experiment was superimposed in the 2017/18 cropping seasons on three years old coffee trees planted on July, 2014. The trial was laid out in an 8X8 simple lattice design with two replications and eight genotypes per each incomplete block (Appendix Figure 1). Each plot consisted of six coffee trees. Spacing were 2mx2m for both between rows and plants in a single row. All the management practices were applied as per the recommendation for the crop.

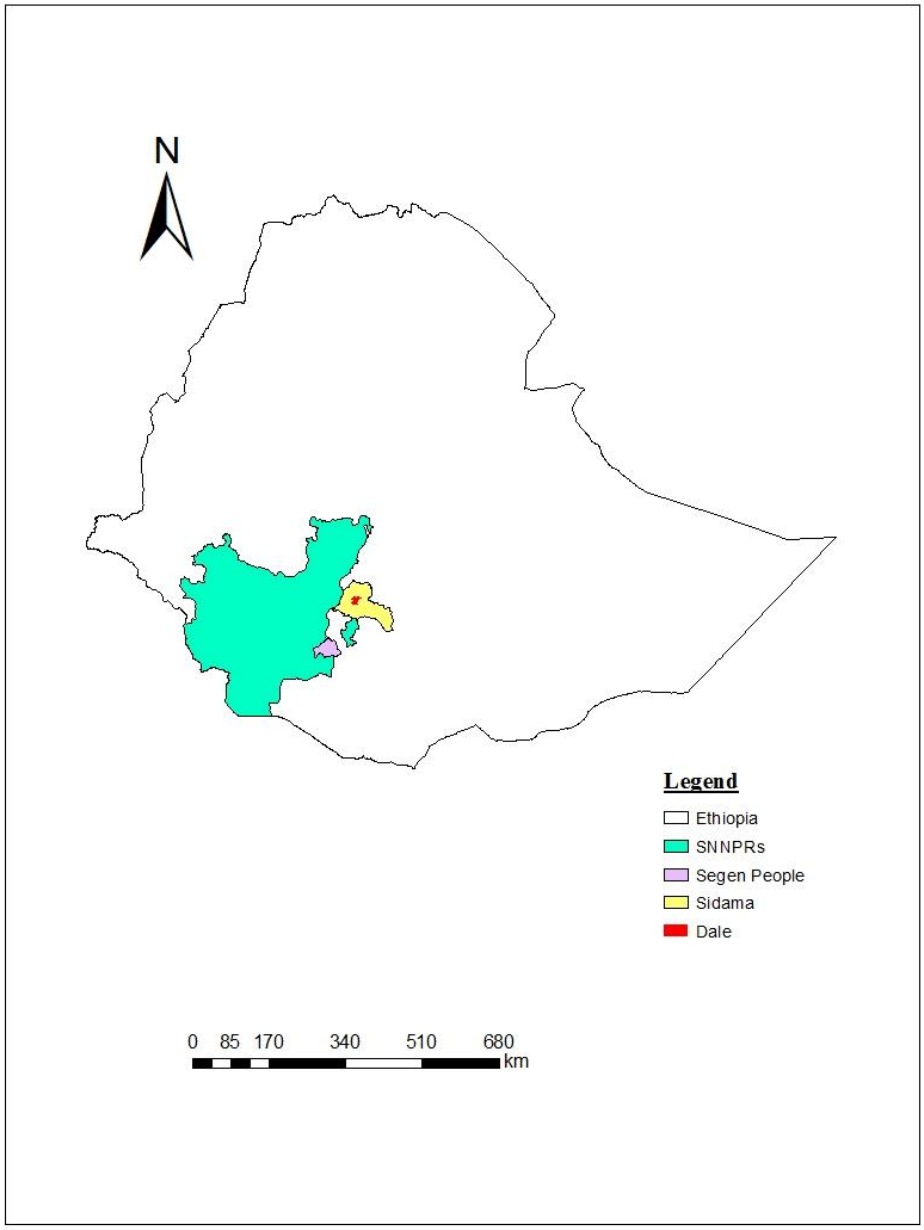

Figure 1 Map of the study site

3.4 Methods of Data Collection

During the course of this study, data on 23 different Agro-morphological characters were collected from four sample trees per row on each accession, and data on 10 qualitative traits were collected, using the standard coffee descriptor of IGPRI (1996) as described below. Coffee berry disease severity and Coffee leaf rust disease severity in percentage were also recorded through visual assessment per four trees.

3.4.1 Quantitative traits

1. **Coffee bean yield (kg/ha):-** weight of fresh cherries in gram per plot were recorded, and converted in to red cherries of coffee in gram per tree (mean of six trees). Clean coffee bean (quantal/ha) = fresh cherries in gram per tree x 0.00417. Clean coffee bean (kg/ha) was calculated as (clean coffee bean (quantal/ha) x 100).
2. **Leaf length (cm):-** average of five normal (node 3 from the terminal bud) leaves were measured from petiole end to apex.
3. **Leaf width (cm):-** average of five normal (node 3 from the terminal bud) leaves were measured at the widest part.
4. **Leaf area (cm^2)** was calculated by multiplying leaf length and width by a constant 0.67
5. **Fruit length (mm):-** average of five normal and mature green fruits were measured at the longest part, using digital caliper.
6. **Fruit width (mm):-** average of five normal and mature green fruits were measured at the widest part using digital caliper.
7. **Fruit thickness (mm):-** average of five normal and mature green fruits were measured at the thickest part using digital caliper.
8. **Seed length (mm):-** average of five normal beans was measured at the longest part.
9. **Seed width (mm):** average of five normal beans were measured at the widest part
10. **Seed thickness (mm):-** average of five normal beans was measured at the thickest part.
11. **Height up to first primary branch (cm):-** height from the ground up to first primary branch was measured, using tape meter.
12. **Total tree height (cm):-** the length from the ground level to the tip of the tree per four trees was measured using tape meter.
13. **Number of main stem node (no):-** the number of nodes from bottom to the top were counted per trees.
14. **Average Inter-node length on orthotropic branch (cm):-** was computed per tree as (TH–HFPB)/TNN-1, where TH = total plant height, HFPB =height up to first primary branch, TNN = total number of main stem nodes (IGPRI, 1996).
15. **Main stem diameter (mm):-** was measured as a diameter of the main stem at five cm above the ground using caliper.
16. **Number of primary branches (no):-** total numbers of primary branches were counted per trees.

17. **Length of primary branch (cm):-** the average length of six selected primary branches (from bottom, middle and top of the tree) was measured using tape meter.
18. **Number of nodes on primary branches (no):-** number of nodes per six selected primary branches (from bottom, middle and top of the tree) were counted and recorded.
19. **Average Inter-node length on primary branches (cm):-** the average internodes length of primary branch was calculated by dividing the average length of primary branch by average number of nodes on primary branch.
20. **Percentage bearing primary branches (%):** was computed per tree as (NBPB/Npb) * 100, where NBPB = number of bearing primary branches per tree, Npb = total number of primary branches per tree
21. **Length of longest primary branch (cm):-** the lengths of longest selected first primary branches were measured using tape meter.
22. **Canopy diameter (cm):-** Was estimated as average length of tree canopy in east-west and north-south direction, using tape meter.
23. **100 Bean weight (g**) calculated as (bean weight at 0% moisture content x 100/ (bean No x 0.89).
24. **Disease data: - Coffee berry disease (CBD**): severity was directly estimated as the percentage of diseased berries (damaged berries over all barriers of bearing branch) from each of the four trees assessed. Additionally, coffee berry disease severity was assessed in different research activities at Awada, Leku and Wonago field conditions to check the performance of standard checks with regard to coffee berry disease reaction (Appendix Table 2).

    Coffee leaf rust (**CLR**): severity percentage of leaves per tree was also directly estimated as the percentage of diseased leaves (damaged leaves over all the top, middle and bottom part of the tree) of four trees assessed.

3.4.2 Qualitative characters

1. **Growth habit:** 1. Open, 2. Intermediate, 3. Compact
2. **Stem habit:** 1. stiff, 2. flexible
3. **Branching habit:** 1 Very few branches (primary). 2 Many branches (primary) with few secondary branches
4. **Angle of insertion on main stem:** 1. Horizontal spreading, 2. Semi- erect

5. **Young leaf tip color:** 1. Light green, 2. Green, 3. Bronze, 4. Light bronze, 5. Redish bronze. The leaf color will be characterized based on the Colour Chart of the Royal Horticultural Society of London (RHS 1966 5th ed.).
6. **Leaf shape:** 1. Ovate, and 2. lanceolate
7. **Leaf apex shape:** 1. Acuminate, 2. Apiculate,
8. **Stipule shape:** 1. Ovate, 2. Triangular, 3. Deltate,
9. **Fruit shape:** 1. Round, 2. Obovate, 3. Elliptic, 4. Obolong
10. **Overall appearance:** 1**.** Elongated conical, 2. Pyramidal and 3. Bushy in overall growth habit.

3.5 Statistical Analysis

3.5.1 Analysis of variance (ANOVA)

All quantitative data was subjected to analysis of variance using the SAS software version 9.3 (SAS, 2014). The normality of each data was check during the analysis using SAS software version 9.3 Shapiro-Wilk W test for normality indicated this assumption, the distribution was normal; except disease data and bean yield. Therefore, coffee bean yield, coffee berry disease and coffee leaf rust were subjected to data transformation before analysis. The arcsine transformation method was used for coffee berry disease and coffee leaf rust, whereas Log transformation was used for coffee bean yield in $kgha^{-1}$ (SAS, 2014). Analysis of Variance for 8 X8 simple lattice designs were done using the mean of sample data for the characters and mean comparisons among accessions were conducted at 5% levels of significance. The 8 X8 simple lattice design analysis of variance was used to derive variance components as structured in Table 2 (Cochran and Cox, 1957).

Table 2 Analysis of variance table for simple lattice design

Sources of variation	D.F	SS	MS	F-value
Replications	r-1	SSR	MSR	$MS_{R/}MSe$
Genotypes	K^2-1	SSG	MSG	$MS_{G/}Mse$
Blocks in rep (adj.)	r(k-1)	SSB	MSB	$MS_{B/}MSe$
Genotype (unadj.)	K^2-1	SSG	MSG	$MS_{G\,/}MSe$
Intra block error	(k-1)(rk-k-1)	SSE	MSE	
Total	rk^2-1	TSS		

Where; r = the number of replication, G = number of genotypes, k = block sizes, SS_R and MS_R are sums of squares and mean squares of replication, respectively; SS_G and MS_G are sums and mean squares of genotypes, respectively; SS_b and MS_b are sums and mean squares of blocks within replication respectively: SSe and MSe are sum and mean squares of intra-block error, respectively and SS_t is sum of squares of the total. The simple lattice design model is presented as follows:

$$\mathbf{y_{ijklm} = \mu + t_i + \beta_j + \chi_k + y_l + \pi_m + \Sigma_{ijklm}}$$

Where,

Y_{ijklm} = response of Y trait from the i^{th} accession, j^{th} replication

μ= overall mean effects

t_i= effects of i^{th} level of treatments

β= effects of j^{th} level of replication

χ_k= effects of K^{th} level of blocks within replications (adjusted for treatments)

y_l = effects of l^{th} level of intra block error.

π_m= effects of the m^{th} randomized complete block error

Σ_{ijklm}= is a random error component

Statistical analyses$Yi = \beta_0 + \beta_1 X_i + b_i + \varepsilon_i$. Relative efficiency (R.E.) of simple lattice over RCBD was also done by subjecting to SAS software.

3.5.2 Estimation of genetic parameters

Different genetic parameters including genotypic variance ($\sigma^2 g$), phenotypic variance ($\sigma^2 p$), phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were estimated using the formula adopted from Burton and De Vane (1953).

$$\mathbf{\sigma^2 p = \sigma^2 g + \sigma^2 e}$$

Where, $\sigma^2 p$ = phenotypic variance, $\sigma^2 g$ = genotypic variance, $\sigma^2 e$ = environmental variance

Genotypic variance (σ2g

Where, $\sigma^2 g$= Genotypic variation, MSg = mean square of genotype, MSe = mean square of error and r=replications

Phenotypic coefficient of variation **= X 100**

Genotypic coefficient of variation **(GCV) =X100**

Where:

σ^2p =Phenotypic variance, σ^2g= Genotypic variance and Grand mean

GCV and *PCV* values were categorized as *low* (0-10%), *moderate* (10-20%) and *high* (20% and above) values, as indicated by Burton and De Vane (1953).

3.5.3 Estimation of heritability and genetic advance

Broad sense heritability values were estimated based on the formula of Falconer and Mackay (1996) as followed:

Heritability in broad sense (H^2b) = x 100. Where, H =heritability in broad sense

3.5.4 Genetic advance under selection (GA)

Then, the genetic advance for selection intensity (k) at 5% (2.06) was estimated by the following formula **(**Allard, 1960).

GA = K ‧ pH

Where, H = Heritability in broad sense, ‧p = Phenotypic standard deviation on mean basis

GA= Expected genetic advance, k = the standardized selection differential at 5% selection intensity (K = 2.063).

Genetic advance as percent of mean was calculated to compare the extent of predicted advance of different traits under selection, using the following formula

GAM = X 100 (Falconer and Mackey, 1996).

Where, GAM=Genetic advance as percent of mean, GA=Genetic advance under selection and $\bar{x}$=Grand Mean of the population

3.5.5 Correlation analysis

Correlation coefficient (r) is a measure of the association between two or more than two variables. The phenotypic correlation and genotypic correlation coefficients between two variables, including genotype were estimated, as described by Singh and Chaudhary (1985).

σg_{xy}= Where:

σg_{xy} = genotypic covariance between traits x and y, *MSPg* = genotypic mean sum product of traits x and y, *MSPe* = environmental mean sum product of traits x and y and

r = number of replication.

$\sigma p_{xy} = \sigma g_{xy} + \sigma e_{xy}$ Where:

σp_{xy} = phenotypic covariance between traits x and y, σg_{xy} = genotypic covariance between traits x and y, σe_{xy} = environmental covariance between traits x and y.

Correlation coefficients at genotypic level (rg_{xy}) were calculated as;

$rg_{xy} = (\sigma g_{xy})/$ Where:

rg_{xy} = genotypic correlation coefficient between traits x and y, σg_{xy} = genotypic covariance between traits x and y, $\sigma^2 \boldsymbol{g}_x$ = genotypic variance of trait x, $\sigma^2 \boldsymbol{g}_y$ = genotypic variance of trait y.

Correlation coefficients at phenotypic level (rp_{xy}) were calculated as;

$rp_{xy=} (\sigma p_{xy})/$ Where:

rp_{xy} = phenotypic correlation coefficient between traits x and y, σp_{xy} = phenotypic covariance between traits x and y, $\sigma^2 \boldsymbol{p}_x$ = phenotypic variance of trait x and $\sigma^2 \boldsymbol{p}_y$ = phenotypic variance of trait y.

3.5.6 Path coefficient analysis

Path coefficient analysis was calculated as suggested by Dewey and Lu (1959) to determine direct and indirect effects of different variables on grain yield as:

$rij = Pij + \Sigma rikPkj$

Where;

rij is mutual association between the independent trait (i) and dependent trait (j) as measured by the correlation coefficient Pij is component of direct effects of the idependent trait (i) on the dependent variable (j); ΣrikPkj is summation of components of indirect effect of a given dependent trait via all other independent traits.

The residual effect (U) was calculated using the formula:

Where: - $=\Sigma pij\ rij$

pij = component of direct effects of the independent character (i) on the dependent character (j) as measured by the path coefficient.

rij = mutual association between the independent character (i) and dependent character (j) as measured by the correlation coefficient.

3.5.7 Cluster analysis

Cluster analysis was used to group the genotypes in to homogeneous forms based on quantitative and qualitative characters. Hierarchical clustering was employed using the

similarity coefficients among the 64 coffee accessions. Clustering was performed using the proc cluster procedure of SAS software by employing the method of average linkage clustering strategy of the observation. The numbers of clusters were determined following the approach suggested by Copper and Miligan (1988) by looking into three statistics, namely: Pseudo F, Pseudo t^2 and cubic clustering criteria using SAS 9.3 software. Genetic divergence between clusters was determined using the generalized Mahalanobis's D^2 statistics (Mahalanobis, 1936) using the equation:

The generalized genetic distance between clusters can be calculated as

$D^2p = (Xi - Xj)\ S^{-1}(Xi - Xj)$.

Where: D^2p= the distance between any two groups i and j. Xi and Xj = the p mean vectors of accessions i and j, respectively. S^{-1} = the inverse of the pooled covariance matrix, and the distance obtained was tested using tabulated F table at 5% level significance.

The D^2 value obtained for pairs of clusters were considered, as the calculated value of Chi-squire (X^2) and were tested for significance at the required level of probability against the tabulated values of X^2 for p degrees of freedom, where p is the number of characters considered (Singh and Chaudhary, 1987).

3.5.8 Shannon Index (H')

Shannon Index (H') was used to analyze the phenotypic diversity of coffee geremplasm accessions depending on the qualitative traits that were recorded. It was calculated using the formula,

$$H = -\ , \quad E_H = H/H_{max} = H/\ln S$$

$$i=1$$

Where S is the number of traits category, E_H is Shannon's equitability, H is Shannon diversity index and pi is the relative proportion of the total number of entries (N) in the i^{th} class (Shannon, 1948).

3.5.9 Principal component analysis (PCA)

This analysis is variable minimizing procedure and is useful, when there is data on a number of variables; the principal components also give a new set of linearly combined measurements and to identify the traits contributing large part of the total variation among the accessions. It was performed using correlation matrix using SAS version 9.3 (SAS, 2014). PCs with Eigen values greater than one were considered as important component

for the total variations. The objective of this analysis was to reduce the observed variables in smaller number of principal component that accounts for most of the variance in the observed variables.

4 RESULTS AND DISCUSSION

4.1 Analysis of Variance

Analysis of variance (ANOVA) of 25 quantitative characters revealed that there was significant difference ($P<0.05$) among the accessions for most of the measured quantitative characters including clean coffee yield, during the 2017/18 cropping season (Table 3). The mean square showed that there was highly significant difference ($P<0.01$) among the coffee accessions for characters like: coffee bean yield, plant height, height up to first primary branch, main stem diameter, canopy diameter, number of bearing primary branches, fruit width, fruit length, bean thickness, bean width, leaf width, 100-coffee beans weight, coffee berry disease and coffee leaf rust. On the other hand, the traits that showed significant ($P<0.05$) level of difference includes:- average internode length of main stem, length of first longest primary branch, number of primary branches, bean length and leaf size.

This indicates the existence of substantial amount of variability among the tested genotypes selection that can be exploited to identify the best performing genotypes for future use. This result is in agreement with many finding (Bayetta, 2001; Atinafu *et al.,* 2013 and Beksisa *et al.,* 2016) who reported variability for the performance of different coffee genotypes for their agronomic traits. The variability present for important traits in the present study clearly proved the possibility to bring considerable improvement mainly in coffee yield and resistance level to leaf rust through selection and hybridization.

The significant difference observed for measured quantitative traits in this study were in agreement with the finding of earlier authors who reported considerable genetic variability within the Arabica coffee population for yield, disease resistance and growth characters. Mesfin and Bayetta (2008) reported the existence of difference among 100 Hararge coffee accessions using 14 quantitative characters. Similarly, Olika *et al.,* (2011b) found that significant difference for 22 quantitative characters studied on 49 coffee accessions. Bayetta (1997) reported high genetic variability within the Arabica coffee population for yield, CBD resistance and growth characters. In addition, the existence of variability among Arabica coffee accessions was further confirmed by many other researchers who reported significant differences among coffee germplasm collected from major coffee growing regions of Ethiopia (Yigzaw, 2005; Getachew *et al.,* (2013) and Masresha (2018).

Table 3Analysis of variance (Mean squares) for 25 quantitative characters of 64 Arabica coffee accessions at Awada, Southern Ethiopia (in 2017/18).

Traits	Sum Square of Mean					CV (%)	RE (%)	Pvalue
	Replication	Blocks in rep.(ad)(14)	Accession (un adjusted(63)	Accession (adjusted(63	Error			
Coffee bean yield (kg)	323107.50	99628.00	476876.00	420534.20**	106366.60	34.06	98.59	<0.0001
Plant height (m)	6340.80	431.90	437.62	406.10**	179.70	5.84	116.12	0.0018
Height up to first primary branch	0.78	7.23	18.72	15.33**	5.34	9.82	101.94	<0.0001
Stem diameter (cm)	2.20	0.20	0.38	0.36**	0.06	4.82	123.86	<0.0001
Length of longest primary branch (cm)	528.94	56.74	49.31	47.85*	20.82	3.97	121.27	0.0015
Number of primary branch	18.10	22.20	36.50	36.14*	12.24	5.12	107.36	0.0336
Number bearing of primary branch	455.3	32.24	37.00	32.11**	17.10	8.22	108.43	0.0026
Number of main stem node	7.80E-05	6.12	7.20	6.99ns	2.40	4.47	118.4	0.2729
Average Internodes length on main stem (cm)	2.94	0.25	0.28	0.25*	0.14	7.60	107.76	0.0169
Canopy diameter (cm)	309.7	120.9	201.6	191.43**	93.30	4.74	101.43	0.0049
Average length of primary branch (cm)	1292.2	12.72	33.12	24.21ns	17.83	4.83	93.64	0.1332
Number of node per primary branch	38.61	1.96	2.90	2.19ns	1.82	6.60	100.13	0.256
Average internodes length on primary branch (cm)	0.22	0.10	0.06	0.06ns	0.05	5.98	103.01	0.515
Fruit length (mm)	4.54	0.58	1.38	1.27**	0.27	3.31	113.80	<0.0001
Fruit width (mm)	3.99	0.29	0.46	0.34**	0.14	3.30	111.54	0.0007
Fruit thickness (mm)	0.08	0.48	0.34	0.25ns	0.21	3.82	114.2	0.2585

Traits	Sum Square of Mean					CV (%)	RE (%)	Pvalue
	Replication	Blocks in rep.(ad)(14)	Accession (un adjusted(63)	Accession (adjusted(63	Error			
Leaf length (cm)	2.28	0.26	0.31	0.28ns	0.2	3.6	101.93	0.0827
Leaf width (cm)	0.05	0.06	0.11	0.08**	0.04	3.85	102.61	0.0086
Leaf size (cm^2)	50.5	12.04	18.93	15.13*	8.01	6.61	103.47	0.0111
Coffee berry disease (%)	121.7	196.5	658.8	544.3**	107.73	50.33	107.52	<0.0001
Coffee leaf rust (%)	244.2	97.93	127.99	125.9**	30.79	27.45	128.83	<0.0001
Hundred bean weight (g)	31.3	2.14	6.16	5.72**	2.03	9.75	100.06	0.0001
Bean length (mm)	7.01	0.22	.43	0.45*	0.27	5.2	96.5	0.0293
Bean width (mm)	0.19	0.03	0.15	0.13**	0.04	3.1	96.22	<0.0001
Bean thickness (mm)	0.28	0.03	0.09	0.08**	0.04	5.27	93.04	0.0022

4.2 Range and Mean Performance of Accessions

The mean and ranges for the 19 quantitative traits of the 64 accessions are presented in (Table 4). The performance of the accessions ranged widely for bean yield (24.0-2474.5 kg/ha), total plant height (190.8-266.7 cm), coffee berry disease severity (0.0-85.0%), coffee leaf rust severity (2.50.0-46.7%), canopy diameter (184.8-223.8cm), length of longest primary branches (93.4-125.9 cm), number of primary branches (62.0-76.0), hundred bean weight (11.2-19.8 g), number of bearing primary branches (41.0-58.0), average internodes length on main stem(3.60-5.7mm), fruit length(14.0-18.2mm), fruit width(10.1-12.3mm), leaf width(4.9-5.9cm), leaf size(36.0-49.1cm^2), bean length(5.5-7.1mm), bean thickness(3.1-4.3mm) and bean width (5.9-7.1mm).

Out of these important traits, highest ranges were obtained for bean yield/kg, CBD severity, total plant height, CLR severity and canopy diameter, which played important role in the total variability of coffee accessions. These high range values for each trait of interest suggest that great opportunity to improve the various desirable traits without much effort through selection as short term strategy and through hybridization as long term strategy. Hence, there is an opportunity to find accessions with disease resistance and high yielding potential among the tested entries that perform better to utilize in coffee improvement program. This result was agreed with the finding of Yigzaw (2005), Olika *et al.* (2011a), Getachew *et al.* (2013), Gizachew *et al.* (2015) and Masresha (2018), who found a wide range of variation for most measured quantitative traits.

The mean performance for bean yield (957.5 kgha^{-1}), total plant height (229.6 cm), CBD severity (18.8%), CLR severity (13.9%), canopy diameter (203.6cm), length of longest primary branches (115.0 cm), number of primary branches (68), hundred bean weight (14.6 g), number of bearing primary branches (50), average internodes length on main stem(4.8cm), fruit length(15.3mm), fruit width(11.2mm), leaf width(5.34cm), leaf size(42.8cm^2), bean length(9.9mm), bean thickness(3.7mm) and bean width (6.4mm). Seven highest mean yields in the studied environment was recorded by accessions AK2, AK10, AK16, AK15, AK38, AK3 and AK5 with respective overall mean yields of 2474.5, 1932.5, 1874.5, 1768, 1729.5, 1620 and1585.5 Kg/ha(Appendix Table 1). This indicated the availability of genetic variability among genotypes in Amaro coffee growing areas. About 24% of the coffee accessions (14), 26% of the coffee accessions (15), 29% of the coffee accessions (17), 33% of the coffee accessions (19) and 76% of the tested coffee

accessions (44) had mean yield exceeding the mean yield of standard check varieties (85257, 971, 7440, 1377 and 974), respectively.

The highest hundred bean weight was recorded by genotypes: 971, AK28, AK36, 85257 and 974 with respective mean values of 19.8, 19.3, 19.2, 17.7 and 17.3 g. The lowest HBW was recorded by genotype AK40, AK39, AK20 and AK22 with12.2, 12.1, 11.3 and 11.2 g respectively. This trait showed a substantial variability among tested accessions. The presence of significant differences among genotypes for 100 bean weight indicates that there is a true genetic difference among genotypes and improving beans size by selection is possible. The significant differences observed among accessions for 100 bean weights illustrates that accessions also plays a significant role to affect bean sizes (weight). The result, therefore, indicates the existing variation among coffee genotypes for bean weight characteristics. This in agreement with other authors who reported that Arabica coffee genetic variation in hundred bean weights (Wintegens, 2004; Yigzaw, 2005; Abrar *et al.*, 2013).

Very lower coffee berry disease severity level of 1.70, 1.65, 1.65, 1.65, 1.65, 0.85, 0.85 and 0.3 % were observed on coffee accession AK10, AK49, AK21, AK48, AK3, AK16, AK53 and AK47, respectively, while accessions AK3, AK53, AK15, AK48, AK23, AK9 and AK52 showed lower levels of coffee leaf rust disease severity 2.5, 2.7, 3.1, 3.4, 3.5, 3.6 and 3.8 %, respectively (Appendix Table 1). On the other hand, the standard check 74112, 1377, 85257, 971 and 974 showed zero (0%) disease severity level at Awada field condition. About (24) accessions had CLR severity level below ten percent. The severity of the disease was observed at Wonago field condition on 1377 and 974 with respective mean values of 6.8% and 2.2%.

The higher range of variability with respect to coffee berry disease enabled Ethiopian coffee breeders in screening for coffee berry diseases resistant varieties and heterotic hybrid cultivars through crossing (Mesfin and Bayeta, 1984). These indicate that the presence of true genetic differences among accessions for coffee disease severity. Generally, the range and mean performance of the traits studied confirmed the presence of an enormous genetic variability between the tested accessions. Hence, there is an opportunity to find genotypes having disease resistance and high yielding potential among the tested entries that perform better than the existing varieties to utilize for the future coffee improvement program.

4.3 Genotypic and Phenotypic Coefficients of Variation

Estimates of genotypic (GV) and phenotypic variability (PV) are presented in (Table 4). According to Deshmukh *et al.* (1986), phenotypic and genotypic coefficients of variation values greater than 20% are considered as high, whereas values less than 10% are considered to be low and values between 10 and 20% are considered as medium. Accordingly, the highest PCV and GCV were recorded for coffee bean yield, coffee berry disease and coffee leaf rust. Hundred bean weights and height up to first primary branch were characterized by moderate PCV and low GCV values. For most of the traits genotypic coefficients of variation were very close to their corresponding estimates of phenotypic coefficient of variation, suggesting the greater role of the genotype in the expression of these traits. PCV was much higher than GCV for coffee berry disease, coffee leaf rust and coffee bean yield indicating the higher influence the environment has on these traits.

The present finding illustrated that, PCV was higher than GCV for all studied quantitative traits, suggesting the observed variation in the coffee accessions were both the combination of genotypic and environment effect. The extent of the environmental influence on any character is indicated by the magnitude of the differences between the genotypic and phenotypic coefficients of variation. Large differences reflect high environmental influence, while small differences reveal high genetic influence (Akinwale *et al.,* 2011). For most of the traits genotypic coefficients of variation were very close to their corresponding estimates of phenotypic coefficient of variation, suggesting the greater role of the genotype in the expression of these traits. However, PCV was much higher than GCV for coffee berry disease, coffee leaf rust and coffee bean yield indicating the greatest influence of the environment on these traits.

The findings of the present study are comparable with the results of Atinafu *et al.* (2017) who reported that the estimates of PCV and GCV in 124 Sidamo coffee accessions for the 19 quantitative characters ranged from 4.52 to 60.89% and 3.92 to 56.52%, respectively. The author also reported higher PCV (25.8, 60.89 and 43.35 and GCV (21.12, 56.52 and 30.79) values for coffee bean yield, CBD and CLR, respectively. Mesfin and Bayetta (2008) reported that the estimates of PCV and GCV in 100 Hararghe coffee accessions for the 14 quantitative characters ranged from 5.9 to 54.8% and 3.2 to 37.5%, respectively.

Similarly, a previous research conducted on 16 coffee genotypes for 18 quantitative characters revealed that the PCV and GCV ranged from 4.5 to 53.4% and 3.3 to 51.7%, respectively (Yigzaw, 2005). Getachew (2012) also reported high PCV (91.5 and 41.7%) and GCV (62.8 and 22.1%) values for CBD reaction and yield per tree, respectively. Olika *et al.* (2011a) reported lower values of PCV (6.11, 6.31, 4.86, 5.51, 7.23 & 7.21) and GCV (5.31, 5.21, 3.86, 4.42, 5.76 &4.98) for bean length, bean width, fruit length, fruit width, plant height and canopy diameter respectively. Unlike in this result, Atinafu *et al.,* (2017) reported moderate PCV (13.28%, 14.18%, 16.74%, 11.16%, 14.23% & 15.00%) and GCV (11.11%, 12.97%, 15.57%, 13.41% & 13.66) for plant height, stem diameter, number of main stem node, canopy diameter, number of primary branch and number of bearing primary branch respectively.

The slight difference in the ranges of these previous studies and this could be due to the differences in the number of genotype studied, age of coffee and environmental conditions under which the genotypes were tested. From the high GCV values in this study it can be deduced that coffee berry disease reaction, coffee leaf rust reaction and coffee bean yield have high amount of exploitable genetic variability. It also signifies that there is greater potential for favorable advance in selection in these attributes when compared to other characters. The present finding is in agreement with the findings of Olika *et al.* (2011a) and Getachew (2012) who reported high PCV and GCV values for coffee berry disease reaction and yield per tree; moderate PCV and GCV values for height up to first primary branch and hundred bean weights.

4.4 Broad Sense Heritability and Genetic Advance

The estimate of the broad sense heritability for various characters of coffee ranged from 16.0% for coffee fruit thickness to 83.3% for stem diameter (Table 4) below. As suggested by Verma and Agarwal (1982) heritability estimates is low (<20%), medium (20-50%) and high (>50%). The recorded estimates of heritability were high (>50%) for stem diameter (83.3%), coffee berry disease reaction (80.2%), fruit length (78.7%), coffee leaf rust reaction (75.5%), coffee bean yield (74.7%), bean width (69.2%), number of primary branches (66.1%), number of main stem nodes (65.7%), height up to first primary branch (65.2%), hundred bean weight (64.5%), fruit width (58.8%), length of longest primary branch (56.5%), plant height (55.8%) and Canopy diameter (51.3%). A high heritability value indicates these traits were less influenced by the environment in their expression.

Hence, selection based on phenotypic traits is effective. Moderate estimate of the broad sense heritability for Leaf width (50.0%), bean thickness (50.0%), leaf size (47.1%), number of bearing primary branches (46.8%), average internodes length on main stem (44.0%), bean length (40.0%) were observed from this study. This implies the possibility of using these traits in coffee improvement through breeding programs, because of good level of correspondence between genotype and phenotype.

These findings are in agreement with the previous work. For instance, Atinafu *et al.* (2017) reported the estimate of the broad sense heritability for various characters of coffee were high for coffee leaf rust reaction, The recorded estimates of heritability were high (>50%) for stem diameter, coffee berry disease reaction, canopy diameter, number of main stem nodes, plant height, number of primary branches, fruit length, hundred bean weight, average bean yield and coffee leaf rust reaction.

Similarly, Bayetta (2001) reported high heritability estimates for all characters measured in his study, and suggested greater effectiveness of selection and improvement to be expected for these characters in the future breeding program. Yigzaw (2005) has also observed high heritability for hundred green bean weight and canopy diameter. In contrast, Getachew (2012) reported moderately low heritability for fruit length, coffee berry disease severity, plant height, average inter node of main stem, leaf length, number of primary branches, average length of primary branches and clean coffee yield per tree. In this study, results are generally in agreement with most of the findings of previous studies.

The estimates of genetic advance as percent of mean (GAM) that could be expected from selecting of the coffee genotypes is presented in (table 6). Johnson *et al*. (1955) stated that genetic advance as the percent of mean was categorized as low (0-10%), medium (10-20%) and high (≥20%). Accordingly, the higher value for GAM for coffee berry disease, coffee leaf rust and bean yield were recorded with respective values of 145.2, 89.3 and 73.8. Moderate GAM recorded on hundred bean weights (15.4%), stem diameter (14.3%) and height up to first primary branch (15.8%). Low GAM were recorded for morphological characters like:- fruit length, leaf width number of main stem node, fruit width, leaf size, leaf length, length of the first longest primary branch, number of node per primary branch, fruit thickness, canopy diameter, average length of primary branch, number of primary branches, number of bearing of primary branches, plant height,

average inter node length of primary branch, average internodes length on maim stem, bean width, bean thickness and bean length (Table 4).

Similarly, Atinafu *et al.* (2017) reported that the GAM was higher for CBD, CLR, average coffee bean yield, and moderate for stem diameter. Unlikely, this author reported moderate GAM for average inter node length of stem, number of primary branches and plant height. Abdi (2009) also reported that the GAM was higher for coffee bean yield per plant and unlikely, for 100 bean weights higher GAM was reported by this author. According to Olika *et al.* (2011a), expected genetic advance as percent of the mean from selecting the top 5% of the genotype were high for height up to first primary branches and yield of coffee, whereas moderate GAM were reported for hundred coffee bean weight, number of primary branch, number of main stem node and leaf width in Arabica coffee accessions.

In addition, Yigzaw (2005) observed relatively high values of genotypic coefficient of variation, broad sense heritability and genetic advance for characters. Furthermore, the combined use of genetic coefficient of variation, heritability and genetic advance seems vital for effective improvement of a particular trait in a population. In this study, high estimates of heritability coupled with high genetic advance as percent of means were observed for characters such as coffee berry disease, coffee leaf rust and bean yield which revealed that most likely the high heritability are due to additive gene effects and improvement through selection based on phenotypic performance can be effective. Since high heritability does not always indicate high genetic gain, heritability with genetic advance considered together might be used in predicting the ultimate effect for selecting superior varieties. Genetic advance gives clear picture and precise view of segregating generations for possible selection. Higher estimates of heritability coupled with better genetic advance confirms the scope of selection in developing new genotypes with desirable characteristics.

Table 4 Estimates of components of variances, coefficient of variances, broad sense heritability (H2 %), expected genetic advance (GA) and genetic advance as percent of mean of Amaro coffee accession at Awada in 2017/18

Traits	Range			Components of variances		Coefficient of variability		H^2 (%)	GA	GAM (%)
	Max	Min	Mean	$(\sigma^2 g)$	$(\sigma^2 p)$	GCV	PCV			
Coffee bean yield (kg)	2474.50	24.00	957.70	157083.80	210267.10	41.38	47.88	74.71	706.72	73.79
Plant height (cm)	266.70	190.80	229.60	113.20	203.05	4.63	6.21	55.75	16.39	7.14
Height up to first primary branch (cm)	31.00	16.70	23.50	5.00	7.67	9.51	11.78	65.17	3.72	15.84
stem diameter (cm)	5.90	4.20	5.10	0.15	0.18	7.59	8.32	83.33	0.73	14.30
Length of longest primary branch(cm)	125.90	93.40	114.98	13.52	23.93	3.20	4.25	56.49	5.70	4.96
Number of primary branch	76.00	62.00	68.30	11.95	18.07	5.06	6.22	66.13	5.80	8.49
Number bearing of primary branch	58.00	41.00	50.30	7.51	16.06	5.45	7.97	46.75	3.86	7.68
Number of main stem node	40.00	32.00	34.80	2.30	3.50	4.35	5.37	65.67	2.53	7.28
Average Internodes length on main stem	5.70	3.60	4.90	0.06	0.13	4.79	7.22	44.00	0.32	6.55
Canopy diameter(cm)	223.80	184.80	203.60	49.07	95.72	3.44	4.81	51.26	10.35	5.08
Average length of primary branch(cm)	98.00	78.30	87.68	3.19	12.11	2.04	3.97	26.35	1.89	2.16
Number of node per primary branch	22.80	17.60	20.50	0.19	1.10	2.10	5.10	16.89	0.36	1.78
Average internodes length on primary branch(cm)	4.70	3.70	4.30	0.01	0.03	1.64	4.03	16.67	0.06	1.38
Fruit length (mm)	18.20	14.00	15.30	0.50	0.64	4.62	5.21	78.74	1.29	8.46
Fruit width(mm)	12.30	10.10	11.20	0.10	0.17	2.82	3.68	58.82	0.50	4.47
Fruit thickness	12.95	11.15	11.98	0.02	0.13	1.18	2.95	16.00	0.12	0.97
Leaf length(cm)	13.05	11.25	12.14	0.04	0.14	1.65	3.08	28.57	0.22	1.82
Leaf width(cm)	5.90	4.85	5.34	0.02	0.04	2.65	3.75	50.00	0.21	3.86
Leaf size(cm^2)	49.10	36.00	42.80	3.56	7.57	4.41	6.43	47.06	2.67	6.24
Coffee berry disease	85.00	0.00	18.80	218.29	272.15	78.59	87.75	80.21	27.30	145.20
Coffee leaf rust	46.70	2.50	13.85	47.56	62.95	49.79	57.29	75.54	12.37	89.28
Hundred bean weight	19.80	11.20	14.60	1.85	2.86	9.30	11.58	64.51	2.25	15.42
Bean length(mm)	33.70	8.50	9.90	0.09	0.23	3.03	4.79	40.00	0.39	3.95
Bean width(mm)	7.10	5.90	6.40	0.05	0.07	3.31	3.98	69.23	0.36	5.69
Bean thickness(mm)	4.30	3.10	3.71	0.02	0.04	3.81	5.39	50.00	0.21	5.56

4.5 Phenotypic and Genotypic Correlation of Coffee Yield with Other Traits

Genotypic (above diagonal) and Phenotypic (below diagonal) correlation coefficients of 19 quantitative traits were computed and presented in (Table 5). The result showed that coefficients of phenotypic correlation were lower than the genotypic correlation coefficients for most of the traits (Table 5) which might be due to less influence of environments on the association of the characters, which might have not weaken the inherent genetic associations. The result agrees with the reports that phenotypic correlations were in most cases lower than the corresponding genotypic values (Abdi, 2009; Olika *et al.,* 2011a; Getachew, 2012; and Beksisa *et al.,* 2017).

Phenotypic correlation: The phenotypic correlation analysis exhibited that clean coffee bean yield in $kgha^{-1}$ was statistically significant and positive with stem diameter, canopy diameter, number of primary branches and number of bearing primary branches with correlation coefficient of (rp=0.30, 0.30, 0.50 and 0.44), respectively (Table 5). This is in agreement with the findings of average bean yield that exhibited significant and positive association with stem diameter, number of primary branches and canopy diameter (Ermias, 2005). However, in contrast to the current results the study by Olika *et al*. (2011a) showed nonsignificant phenotypic correlations of bean yield with all morphological characters.

Genotypic correlation: The genotypic correlation analysis result exhibited that clean coffee bean yield exhibited positive and significant correlation with number of primary branch, number of bearing primary branch, stem diameter and canopy diameter with correlation coefficient (rg=0.70, 0.61, 0.34 and 0.38) respectively (Table 5). These suggest that coffee yield would increase with the increase of these characters. These indicate that greater importance and reliability of these characters for the improvement of yield in coffee. The breeding implication is that selection of one of the characters might result in the improvement of other characters.

In studies of genetic divergence and the processes of evaluation and selection, it is important to maintain traits that are correlated with the majority of traits (Ferrao *et al*., 2008). The close relationship between yield and yield attributing traits might be exploited in selection programme, which might be helpful in developing high yielding genotypes. Thus, breeders might need to emphasize these characters, in selection and crop

improvement program. This finding is in agreement with Yigzaw (2005); Olika *et al*. (2011a) and Beksisa *et al.*, (2017) who reported positive and significant correlation of most of the quantitative characters with yield. Srinivasan (1980) also reported high and positive correlation of stem diameter and length of primary branches with yield. Similarly, Walyaro and Van der Vossen (1979) also reported significant and positive genotypic correlations between yield and stem diameter at the base of the main stem. Walyaro (1983) and Marandu *et al*. (2004) also reported that coffee yield is influenced by important characters, like number of primary branches and canopy diameter.

In general, genotypic correlation coefficients were higher in magnitude than the corresponding phenotypic correlation coefficients for almost all of the characters, indicating that there is a strong inherent association between the characters studied. Higher genotypic correlation might be due to the absence of masking or modifying effect of environment on the genetic association among traits (Johnson *et al.*, 1955). The clean coffee bean yield was significant and negatively correlated with coffee berry disease severity at genotypic level. This implied that through selection of tolerant or resistant coffee accessions, optimum yield of coffee accessions could be achieved in efforts of variety development in selection program. Therefore, it is suggested that independent selection may have to be carried out for improvement of each character.

4.6 Phenotypic and Genotypic Correlation among Morphological Traits

It was observed that, a positive and significant phenotypic and genotypic correlation among characters like: 100 coffee bean weight, bean width, bean length, bean thickness, fruit length, and fruit width had close association with each other. The other traits: plant height, length of longest primary branch, canopy diameter, number of primary branch, number of bearing primary branch and stem diameter had close correlation to each other. The result reveal that, the greater the number of primary branches, the larger will be the number of bearing primary branch, length of the longest primary branch, stem diameter and canopy diameter. Coffee berry disease and coffee leaf rust reaction of tested coffee accessions had also close significant and positive association with each other. This indicated that for those traits which were positively associated the improvement for one trait will simultaneously improve the other. Whereas, those traits, which were negatively correlated the improvement for one trait antagonistically affect the other.

It was found that reaction to coffee berry disease were negative and significantly correlated with 100 bean weight, bean width and bean thickness with phenotypic correlation coefficient values of (rg=-0.257, -0.183 and -0.184), respectively. The number of primary branch (rg=-0.459) and number of bearing primary branch (rg=-0.37) were negatively and significantly correlated with CBD at genotypic level. Therefore, selection for coffee berry disease could negatively affect the improvement of number of primary branch and number of bearing primary branch as these characters showed negative and significant correlation at genotypic correlation coefficients. This implied that, the selection for any one of these characters is not likely to result in improvement of the others. In such a situation, it is suggested that independent selection may have to be carried for improvement of each character. From this study, it was suggested that high yielding population in coffee may be selected by concentrating upon number of primary branch, number of bearing primary branch, length of the longest primary branch and canopy diameter. Since the four traits are correlated among themselves, selection in one of the traits can result in the improvement of the other traits.

Table 5 Genotypic (above diagonal) and phenotype (below diagonal) correlation coefficient among 19 significant characters of Amaro coffee accessions at Awada in 2017/18

Variable	YLD	HBW	BL	BW	BT	FL	FW	LW	LS	CBD	CLR	LLPB	PH	HUFPB	SD	NPB	NBPB	AINL	CD
YLD		0.23	0.11	0.20	0.18	0.22	0.04	-0.03	-0.05	-0.64**	-0.17	0.19	0.07	0.12	0.34**	0.70**	0.61**	0.03	0.38**
HBW	0.18		0.69**	0.59**	0.67**	0.58**	0.57**	-0.04	0.02	-0.24	0.06	-0.03	0.10	0.15	-0.29*	0.13	0.09	0.14	-0.23
BL	0.03	0.66**		0.42**	0.54**	0.67**	0.33**	0.17	0.24	-0.02	-0.13	-0.04	0.22	0.23	-0.12	0.04	0.10	0.287*	-0.12
BW	0.10	0.54**	0.42**		0.75**	0.32**	0.55**	0.21	0.17	-0.17	0.12	0.05	0.20	0.25	-0.18	0.06	0.14	0.23	-0.04
BT	0.07	0.58**	0.59**	0.71**		0.46**	0.57**	0.26*	0.28*	-0.13	0.23	0.17	0.18	0.20	-0.15	0.11	0.17	0.20	-0.03
FL	0.14	0.51**	0.56**	0.31**	0.39**		0.43**	0.16	0.19	-0.15	-0.05	0.13	0.20	0.14	0.08	0.16	0.21	0.21	0.10
FW	-0.04	0.51**	0.37**	0.49**	0.49**	0.46**		-0.03	-0.00	-0.15	0.30*	0.02	-0.00	0.13	-0.07	-0.10	-0.12	-0.02	-0.08
LW	-0.00	-0.04	0.15	0.16	0.24**	0.169	0.01		0.95**	-0.04	-0.21	0.16	0.16	0.03	0.08	-0.18	-0.13	0.33**	0.22
LS	-0.01	-0.04	0.15	0.12	0.23**	0.18*	0.00	0.93**		-0.07	-0.21	0.14	0.13	0.09	-0.01	-0.23	-0.14	0.31*	0.14
CBD	-0.53**	-0.30**	-0.11	-0.18**	-0.18*	-0.13	-0.15	-0.04	-0.05		0.39**	-0.14	-0.02	-0.06	-0.04	-0.46**	-0.31*	0.02	-0.15
CLR	-0.07	0.03	-0.18*	0.05	0.11	-0.05	0.19*	-0.08	-0.07	0.36**		0.06	-0.12	-0.128	-0.16	-0.04	0.06	-0.13	-0.15
LLPB	0.13	0.08	0.12	0.08	0.20*	0.19*	0.07	0.12	0.07	-0.07	-0.00		0.49**	0.09	0.46**	0.33**	0.36**	0.42**	0.65**
PH	-0.01	0.22*	0.31**	0.22*	0.23*	0.27**	0.19*	0.14	0.13	-0.10	-0.12	0.43**		0.33**	0.15	0.35**	0.33**	0.85**	0.32**
HUFPB	0.13	0.15	0.19*	0.21*	0.16	0.15	0.14	0.02	0.05	-0.07	-0.09	0.06	0.24**		0.100	0.09	0.09	0.11	0.18
SD	0.30**	-0.10	0.06	-0.09	-0.05	0.19*	0.06	0.07	-0.02	-0.03	-0.14	0.49**	0.22*	0.13		0.30*	0.28*	0.09	0.73**
NPB	0.50**	0.16	0.108	0.09	0.08	0.18*	-0.04	-0.17	-0.19*	-0.30**	-0.09	0.31**	0.36**	0.09	0.32**		0.83**	0.06	0.38**
NBPB	0.44**	0.18*	0.165	0.10	0.15	0.21*	-0.03	-0.12	-0.15	-0.19	0.01	0.42**	0.34**	0.05	0.36**	0.68**		0.09	0.34**
AINL	-0.09	0.21*	0.34**	0.26*	0.28*	0.24**	0.15	0.29**	0.28**	-0.07	-0.09	0.35**	0.84**	0.04	0.13	0.07	0.10		0.18
CD	0.30**	-0.03	0.06	-0.04	0.06	0.13	-0.03	0.19*	0.13	-0.13	-0.10	0.59**	0.36**	0.12	0.64**	0.34**	0.36**	0.21*	

*, ** = Significant at 5% and 1%, respectively.

Where Yld=Coffee bean yield, Where HUFPB=Height up to first primary branch, SD=stem diameter, NPB=Number of primary branch, NBPB=Number bearing of primary branch, NMSN=Number of main stem node, FL=Fruit length, FW=Fruit width, CBD=Coffee berry disease, CLR=Coffee leaf rust, PH=Plant height, LW=Leaf width, LS=Leaf size, LLPB= length of longest primary branch, AINL=Average Internodes length, CD=Canopy diameter, HBW=Hundred bean weight, BL= Bean length, BT=Bean thickness and BW=Bean width

4.7 Path Coefficient Analysis

As per path coefficient analysis, the highest direct positive effect was shown by average inter node length of main stem (1.08), followed by canopy diameter (0.41), height up to first primary branch (0.30), number of bearing primary branch (0.30), number of primary branch (0.18) and leaf width (0.17) (Table 6). Low magnitude and positive direct effects were recorded for fruit width (0.12), hundred bean weight (0.09), bean width (0.03), stem diameter (0.01) and bean length (0.004). The negative effect were recorded for plant height (-1.25), leaf size (-.0.26), coffee berry disease (-0.23), bean length (-0.06), coffee leaf rust (-0.05), length of the first longest primary branch (-0.19) and fruit length (-0.05). The positive direct effects of stem diameter and number of primary branches in this study were similar with findings of Johnson *et al.* (1955) and Atinafu and Mohammed (2017). Path coefficient analysis revealed that bean width (rg=0.20) and hundred bean weight (rg=0.23) had positive and direct effect on coffee bean yield, though exhibited positive and non-significant genotypic correlation with coffee yield.

These traits, however, had nonsignificant genotypic association with yield and exhibited a substantial indirect counter balance effect via one another, suggesting that the characters had significant contribution for coffee bean yield at genotypic level. Average internodes length on main stem had the highest positive and direct effect on coffee yield, but exhibited negative phenotypic correlation (rp=-0.09) with coffee yield. The negative correlation it showed with coffee yield was mainly due to negative indirect effects via other traits: bean thickness, fruit length, fruit width, leaf size, CBD, length of longest primary branch and plant height. This indicated that restricted simultaneous selection has to be followed; as restrictions are to be imposed to nullify the undesirable indirect effects in order to make use of the direct effect of these traits.

The positive direct effect of stem diameter (0.09), bean width (0.03) and hundred bean weight (0.05) on coffee bean yield agreed with other findings of Masreshaw (2018). Similarly, Masreshaw (2018) also reported that negative direct effect of yield contributing traits like: total plant height (-0.08), bean length (-0.31), coffee berry disease (-0.16) and coffee leaf rust (-0.06). In contrast, the findings of this study revealed positive and direct effect of number of primary branches, fruit width (0.07) and negative effect of canopy diameter (-0.06) on coffee bean yield (Table 6). The other scholar found that the positive

highest direct effect on coffee yield was exerted by plant height (1.56) and canopy diameter (1.56**).**

Average internodes length (-1.86) and number of primary branches (-1.80) also exerted high negative effects on yield (Beksisa *et al.,* 2017). Ermias (2005) reported positive direct effect of plant height but negative direct effects of canopy diameter on yield. Atinafu and Mohammed *(*2017) observed direct positive effect by plant height, hundred bean weights, coffee berry disease, stem diameter and average length of primary branches, number of primary branches, number of main stem nodes and bean yield. On the contrary, plant height (-1.25), fruit length (-0.05), bean thickness (-0.05) and length of longest primary branch (-0.19) had negative direct effect and positive genotypic correlation coefficients of (rg=0.07, 0.22, 0.18 and 0.19), respectively on coffee bean yield. Hence, the positive correlation coefficient was largely due to their respective positive indirect effects of other characters.

The main selection criterion in coffee is yield, quality and disease resistance. Other agronomic characters related to yield potential have been studied to increase the indirect selection efficiency. In this study, the positive direct effect on coffee yield was exerted by average inter node length of main stem, canopy diameter, height up to first primary branch, number of bearing primary branch, number of primary branch and leaf width, fruit width, hundred bean weight, bean width, stem diameter and bean length. This indicates that, with other characters kept constant, direct selection on the basis of average inter node length of main stem, number of main stem nodes, canopy diameter, height up to first primary branch, number of bearing primary branch, number of primary branch and leaf width, fruit width, hundred bean weight, bean width, stem diameter and bean length would be much effective for the improvement of coffee yield. This is usually happens and they are well known as the most important characters that influence the coffee yield directly.

The residual effect permits precise explanation about the pattern of interaction of other possible components of yield. In other words, residual effect measures the role of other independent variables which were not included in the study on the dependent variable. In this study, the estimated residual effect was 0.35 indicating that about 65% of the variability in yield was contributed by the characters studied in path analysis. This residual effect towards yield in this study might be mainly due to the other characters which were

not included in the investigation and environmental factor. Therefore, the aspect of intensive germplasm exploration in the Amaro coffee considering additional characters was suggested in order to confirm the results. In general, the path analysis carried out in the present study revealed that the main components of bean yield which had positive direct effect of bean yield should be given high priority for making selection for high yielding accessions in Amaro coffee accessions.

Table 6 Direct and indirect effects of bean yield and 18 yield contributing characters of Amaro coffee accessions at Awada in 2017/18

Variable	HBW	BL	BW	BT	FL	FW	LW	LS	CBD	CLR	LLPB	PH	HUFPB	SD	NPB	NBPB	AINL	CD	rG
HBW	**0.085**	0.003	0.018	-0.037	-0.031	0.067	-0.007	-0.004	0.054	-0.003	0.006	-0.128	0.044	-0.002	0.023	0.027	0.152	-0.095	0.229
BL	0.059	**0.004**	0.013	-0.030	-0.036	0.039	0.029	-0.061	0.004	0.006	0.008	-0.280	0.070	-0.001	0.008	0.028	0.311	-0.051	0.109
BW	0.051	0.002	**0.030**	-0.042	-0.017	0.064	0.034	-0.044	0.038	-0.006	-0.009	-0.253	0.075	-0.002	0.010	0.040	0.248	-0.018	0.198
BT	0.057	0.002	0.022	**-0.056**	-0.025	0.067	0.044	-0.073	0.030	-0.011	-0.032	-0.225	0.060	-0.001	0.020	0.050	0.216	-0.011	0.179
FL	0.049	0.003	0.010	-0.026	**-0.054**	0.050	0.026	-0.049	0.035	0.002	-0.024	-0.246	0.041	0.001	0.028	0.060	0.226	0.040	0.216
FW	0.049	0.001	0.016	-0.032	-0.023	**0.117**	-0.005	0.001	0.033	-0.014	-0.004	0.004	0.038	-0.001	-0.017	-0.035	-0.020	-0.034	0.043
LW	-0.004	0.001	0.006	-0.015	-0.008	-0.004	**0.167**	-0.245	0.009	0.010	-0.031	-0.198	0.010	0.001	-0.031	-0.039	0.359	0.092	-0.030
LS	0.001	0.001	0.005	-0.016	-0.010	-0.001	0.159	**-0.257**	0.015	0.009	-0.027	-0.160	0.028	0.000	-0.040	-0.042	0.338	0.059	-0.045
CBD	-0.020	0.000	-0.005	0.008	0.008	-0.017	-0.007	0.017	**-0.227**	-0.018	0.027	0.024	-0.019	0.000	-0.081	-0.090	0.018	-0.062	-0.644
CLR	0.005	-0.001	0.004	-0.013	0.003	0.035	-0.035	0.053	-0.088	**-0.046**	-0.011	0.144	-0.038	-0.001	-0.007	0.017	-0.138	-0.064	-0.165
LLPB	-0.003	0.000	0.001	-0.010	-0.007	0.002	0.027	-0.036	0.032	-0.003	**-0.191**	-0.614	0.026	0.004	0.058	0.105	0.452	0.270	0.189
PH	0.009	0.001	0.006	-0.010	-0.011	0.000	0.026	-0.033	0.004	0.005	-0.093	**-1.254**	0.099	0.001	0.061	0.096	0.916	0.134	0.065
HUFPB	0.012	0.001	0.007	-0.011	-0.007	0.015	0.006	-0.024	0.014	0.006	-0.016	-0.416	**0.300**	0.001	0.015	0.025	0.115	0.075	0.117
SD	-0.025	-0.001	-0.005	0.008	-0.004	-0.008	0.013	0.003	0.008	0.007	-0.088	-0.185	0.030	**0.008**	0.052	0.083	0.092	0.303	0.335
NPB	0.011	0.000	0.002	-0.006	-0.008	-0.011	-0.030	0.059	0.104	0.002	-0.063	-0.434	0.026	0.002	**0.176**	0.243	0.068	0.158	0.704
NBPB	0.008	0.000	0.004	-0.010	-0.011	-0.014	-0.022	0.036	0.070	-0.003	-0.068	-0.409	0.025	0.002	0.146	**0.294**	0.096	0.139	0.613
AINL	0.012	0.001	0.007	-0.011	-0.011	-0.002	0.056	-0.080	-0.004	0.006	-0.080	-1.061	0.032	0.001	0.011	0.026	**1.083**	0.072	0.029
CD	-0.020	-0.001	-0.001	0.002	-0.005	-0.010	0.037	-0.037	0.034	0.007	-0.124	-0.405	0.054	0.006	0.067	0.099	0.189	**0.414**	0.376

Residual=0.35

Where HUFPB=Height up to first primary branch, SD=stem diameter, NPB=Number of primary branch, NBPB=Number bearing of primary branch, NMSN=Number of main stem node, FL=Fruit length, FW=Fruit width, CBD=Coffee berry disease, CLR=Coffee leaf rust, PH=Plant height, LW=Leaf width, LS=Leaf size, LLPB= length of longest primary branch, AINL=Average Internode length, CD=Canopy diameter, HBW=Hundred bean weight, BL= Bean length, BT=Bean thickness and BW=Bean width

4.8 Genetic Divergence Study

4.8.1 Cluster analysis of genotypes using quantitative traits

The phenotypic similarities of 64 coffee accessions were done using cluster analysis based on 19 significant quantitative characters. Cluster analysis confirmed the presence of variation among Amaro coffee accessions. The 64 coffee accessions were grouped into five clusters (Table 7). The genotypes used as checks, 1377, 7440, 971 and 85257 were grouped in cluster I; whereas genotypes 74112 and 974 were grouped in cluster II. The majority of accessions 59 (92.20%) were classified in to three clusters 26, 26 and 7 accessions in clusters I, II and IV, respectively. Cluster III and V had 4 and 1 members, respectively. Large number of accessions were grouped in to Cluster I and II, which contained 26 accessions (40.63%) and followed by IV and IV with 7 (10.94%) and 4 (6.25%) accessions respectively. Whereas, cluster V contained one accession (1.56%) of the total population, indicate that coffee accessions of the same group were morphologically similar.

The clustering pattern showed that accessions collected from different Kebeles clustered together in the same group, for instance, accessions collected from all Kebeles clustered together in cluster I and II. This finding is in line with Seyoum (2003) who reported that accessions collected from different geographic origins were clustered together in the same group. In addition, in the present study, accessions collected from the same Kebeles were clustered into different clusters, suggesting the existence of high genetic diversity in accessions obtained within each peasant associations. So, this diversity could be exploited through further breeding to broaden the genetic base of the crop and develop new varieties. Abdi (2009) also reported phenotypic diversity among 49 Hararge coffee accessions for 16 quantitative characters and found out that the accessions were grouped into 6 clusters. Similarly, Olika *et al.* (2011b) has made cluster analysis based on 22 quantitative traits grouped 49 Limmu coffee genotypes in to four clusters. The 124 coffee genotypes were grouped into ten clusters (Atinafu *et al.*, 2017). Generally, accessions grouped in different clusters are expected to be more divergent than within grouped individuals.

Table 7 Distribution of Amaro coffee accessions into five clusters based on D2 analysis for 64 coffee accessions at Awada (2017/18).

Cluster No.	Number of Accessions	Percent	Accessions
I	26	40.63	AK50, AK55, AK54, AK56, AK45, AK57, AK41, AK28, AK9, AK12, 1377, AK58, 85257, AK46, AK13, AK35, AK47, AK21, AK6, 971, AK18, 7440, AK34, AK26, AK51 & AK49
II	26	40.63	AK31, AK44, AK20, AK37, AK8, AK30, AK48, AK27, AK39, AK32, AK52, AK24, AK29, AK1, AK11, AK7, AK36, AK25, AK33, AK4, AK14, 974, 74112, AK19, AK40 & AK53
III	4	6.25	AK17, AK22, AK43 & AK42
IV	7	10.94	AK5, AK23, AK15, AK38, AK3, AK10 & AK16
V	1	1.56	AK2

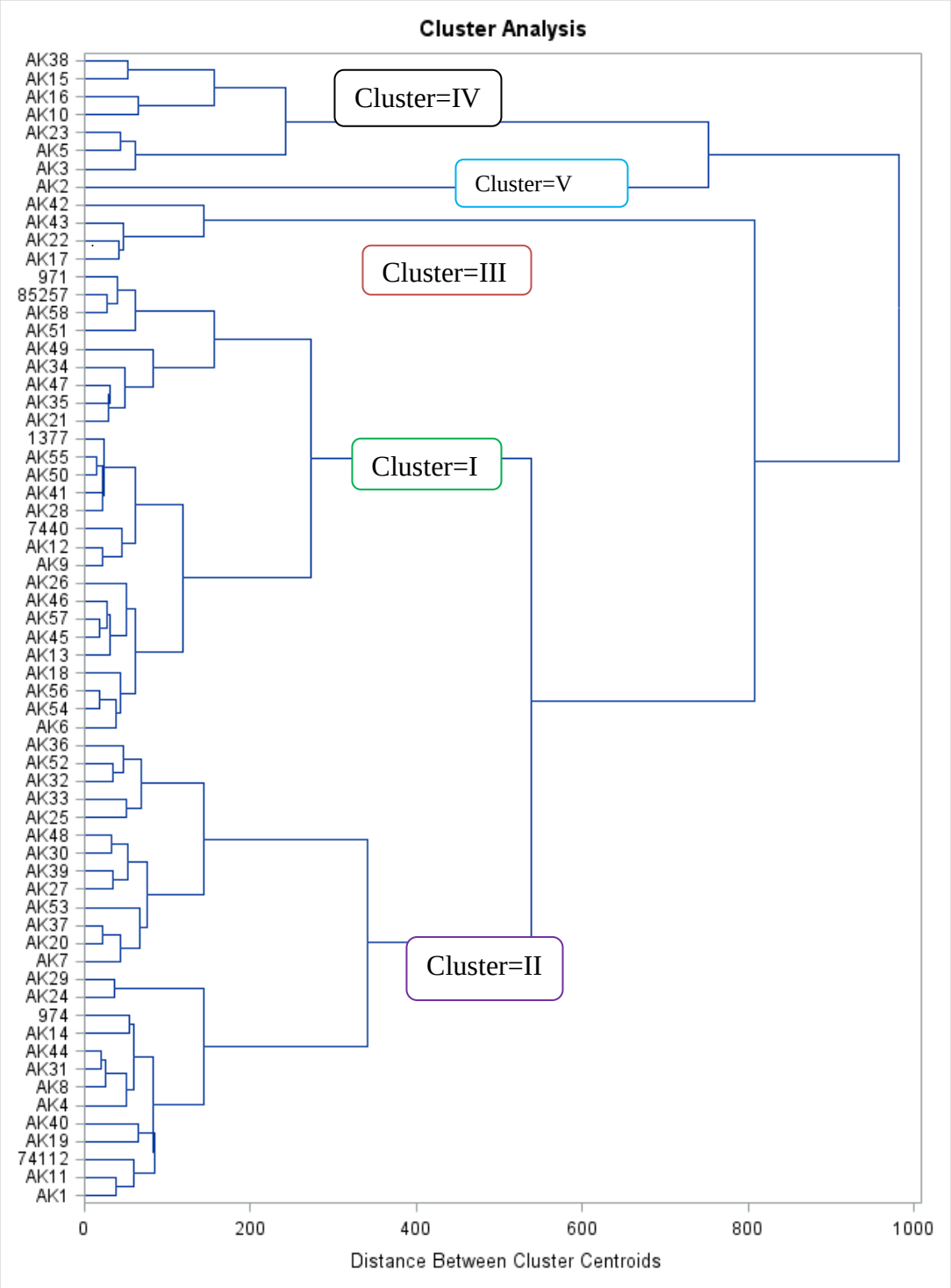

Figure 2 Tree diagram of 64 Arabica coffee accessions using 19 coffee quantitative traits

4.8.2 Cluster analysis based on qualitative characters

Cluster analysis was also confirmed the presence of variation among 64 coffee accessions based on qualitative traits. Accordingly, Amaro coffee accessions were grouped into six distinct groups (Table 8). Cluster-I was the largest and consisted of 33 accessions (51.6%) followed by cluster-II (31.3%), cluster-III (7.8%), cluster IV (4.7%), V (3.1%) and cluster

VI (1.6%). Accessions grouped in cluster I have predominately triangular stipule shape and lanceolate leaf shape. Cluster II on the other hand, comprised of 20 accessions and characterized by lanceolate leaf shape. In addition, five accessions were grouped under cluster III. These accessions predominantly possess typically open growth habit with spreading branch, flexible stem, bronze young leaf tip color and ovate stipule shape. Cluster IV was characterized by apiculate leaf apex shape, flexible stem, deltate stipule shape, elliptic fruit shape and many branches (primary) with few secondary branches. One accession is grouped in cluster VI (AK40) was characterized by open growth habit, strong stem, elliptic fruit shape, many branches (primary) with many secondary branches, ovate leaf shape, spreading angle of insertion on primary branch and bushy type in overall appearance. Cluster V had compact growth habit, semi-erect of insertion on primary branch, flexible stem, acuminate leaf shape, apiculate leaf apex and pyramidal in overall appearances. Atinafu *et al.* (2017) also clustered about 124 Sidama coffee accessions into 10 distinct groups based on seven qualitative traits. Similarly, the 19 coffee accessions were classified into five distinct groups (Tounekti *et al.,* 2017).

Moreover, the agro-morphological characters among evaluated Amaro Arabica coffee accessions have confirmed the presence of diversity. Hence, the existence of genetic diversity is potential resource for improvement of coffee through selection and hybridization. The coffee germplasm should be properly conserved and might serve as raw material for future coffee genetic improvement program. In addition of the observed variability for very important traits in coffee quality attributes and disease resistance related traits might be exploited, which could help to improve the productivity of coffee. The morphological diversity observed in this study should be further characterized using molecular techniques (DNA markers).

Table 8 Clustering patterns of 64 coffee accessions based on ten qualitative characters

Cluster No.	Number of Accessions	Percent	Accessions
I	33	51.6	AK15, AK32, AK50, AK49, AK4, AK5, AK1, AK13, AK7, AK34, AK42, AK14, AK52, 974, AK27, AK20, AK23, AK3, AK54, K56, 74112, AK31, AK26, AK6, AK39, AK38, AK17, AK12, AK2, K36, AK28, AK48, 7440
II	20	31.3	AK46, AK30, AK47, AK57, AK22, AK44, AK41, AK25, AK21, AK24, AK43, AK51, 971, AK53, AK19, AK55, AK8, AK58, AK11, AK10
III	5	7.8	AK16, AK9, AK45, AK29, AK35
IV	3	4.7	AK33, 1377, AK37
V	2	3.1	85257, AK18
VI	1	1.6	AK40

4.8.3 Cluster mean analysis

The mean value of the 19 quantitative characters in each cluster is presented in (Table 9). Cluster means analysis revealed appreciable variation for various characters. A close look into the cluster mean revealed that clusters differ with respect to different characters. Cluster V consisted of one accession having characteristic feature of relatively shorter average internodes length on main stem (4.4cm), longer length of the longest primary branches (118.5), less subjected to coffee leaf rust (9.7%), short bean length (9.3mm), short bean width (6.2mm), short bean thickness (3.6mm), short fruit width (10.7mm) and light 100 bean weight (12.8g). Moreover, this cluster is characterized by high yield (2474.5 $kgha^{-1}$), shorter plant height (210cm), relatively longer height up to first primary branch (24.2cm) and wider stem diameter (5.9cm) as compared to other cluster.

Cluster IV, which contained seven accessions known for its unique characteristics of having high number of primary branch (70.6), high number of bearing primary branch (53.1), relatively wider bean thickness (3.7mm), high coffee bean yield (1722.1 $kgha^{-1}$), shorter height up to first primary branch (23.1cm) and relatively tolerant to coffee berry disease (11.72). Cluster III was characterized by low number of primary branch (57.1), low number of bearing primary branch (40.5), relatively more susceptible to both coffee berry disease (61.9%) and coffee leaf rust (16.9%), wide leaf area (44.3cm^2), narrow bean

thickness (3.6mm), narrow fruit length (14.8mm), shorter stem diameter (4.6cm), lower coffee bean yield (86.0 $kgha^{-1}$) and wider fruit width (11.3mm). Cluster II was characterized by shorter length of the longest primary branch (112.7cm), narrow bean thickness (3.7mm), narrow leaf width (5.2cm) and narrow leaf size (41.6). Accessions in cluster I consisted of about 26 coffee accessions with longer average internodes length on main stem(5.0cm), long plant height (232.0cm), heavier 100-bean weight (15.2g), longer bean length (10.0mm), wider bean width (6.5mm), long fruit length (15.6mm) and wide fruit width (11.3mm).

Intercrossing between genotypes of these diverse clusters would generate a broad spectrum of variability for effective selection in the segregating generations for the development of high yielding cultivars (Singh *et al.*, 1987). However, it is important to note that in calculating cluster mean, the superiority of a particular genotype with respect to a given character might be diluted by other genotypes that are related and grouped in the same cluster which are inferior or intermediary for that character under consideration. Hence, high inter cluster distance indicated that there is a high chance for obtaining transgressive segregates and maximizing heterosis by crossing accessions belonging to different clusters as there is a higher chance that distinct accessions would contribute by unique desired alleles at different loci (Ghaderi *et al.*, 1884).

Table 9 Cluster mean value of 19 significant quantitative characters for five clusters of Amaro coffee accessions at Awada in 2017/18

Variable	Cluster means					Cluster mean differences				
	I	II	III	IV	V	I	II	III	IV	V
YLD	1161.40	623.87	86.00**	1722.14	2474.50**	203.74	-333.80	-871.66	764.48	1516.84
PH	232.02**	229.17	226.55	227.08	210.00*	2.39	-0.46	-3.08	-2.55	-19.63
HUFPB	23.76	23.32	24.00	23.14*	24.15**	0.22	-0.22	0.46	-0.40	0.61
SD	5.16	4.97	4.64*	5.31	5.85**	0.08	-0.11	-0.44	0.23	0.77
CD	207.32	199.28	194.26*	210.25	211.45**	3.70	-4.35	-9.36	6.63	7.83
AINL	4.97**	4.82	4.88	4.79	4.40*	0.10	-0.06	0.00	-0.08	-0.47
NBPB	50.98	50.29	40.50*	53.07**	52.00	0.69	0.00	-9.79	2.78	1.71
NPB	69.42	68.33	57.13*	70.57**	70.00	1.08	-0.02	-11.22	2.23	1.66
LLPB	116.73	112.68	113.43	117.44	118.50**	1.75	-2.30	-1.56	2.46	3.52
CBD	8.71	25.92	61.89**	6.48*	7.50	-10.06	7.16	43.12	-12.29	-11.27
CLR	14.00	14.13	16.86**	11.14	9.65*	0.15	0.28	3.02	-2.71	-4.20
HBW	15.18**	14.42	13.78	14.16	12.80*	0.55	-0.21	-0.86	-0.48	-1.84
BL	10.02**	9.94	9.73	9.79	9.34*	0.09	0.01	-0.20	-0.14	-0.59
BW	6.54**	6.36	6.36	6.33	6.16*	0.12	-0.07	-0.06	-0.09	-0.26
BT	3.78**	3.68	3.62	3.65	3.59*	0.06	-0.03	-0.09	-0.06	-0.13
FL	15.61**	15.13	14.78*	15.18	15.05	0.31	-0.17	-0.53	-0.13	-0.25
FW	11.30**	11.11	11.26	11.05	10.70*	0.12	-0.07	0.08	-0.13	-0.48
LW	5.41	5.25*	5.43**	5.39	5.35	0.07	-0.09	0.09	0.05	0.01
LS	43.48	41.59*	44.31**	43.95	43.35	0.66	-1.22	1.50	1.13	0.53

Where, * is the lowest cluster mean, ** is the highest cluster mean difference, YLD=Coffee bean yield, NPB=Number of primary branch, NBPB=Number bearing of primary branch, NMSN=Number of main stem node, FL=Fruit length (mm), FW=Fruit width (mm), CBD=Coffee berry disease, CLR=Coffee leaf rust, PH=Plant height, SD=Stem diameter (cm), LLPB=Length of longest primary branch(cm), AINL= Average internode length (cm), CD=Canopy diameter (cm), HBW=Hundred bean weight (gm), BL=Bean length (mm), BW=Bean width (mm)

4.8.4 Inter-cluster distance (D^2) analysis based on quantitative traits

The chi-square test for the five clusters indicated that there were highly significant differences (P<0.01) among each other (Table 10). The smallest inter-cluster distance (D^2=23.62) was observed between clusters I and IV while the highest (D^2=371.29) was between clusters III and V. In most cases, the accessions among the clusters are significantly (P<0.01, x^2=34.80) divergent from each other. Maximum inter cluster distance was observed between cluster III and V (D^2=371.29), followed by between clusters II and V (D^2=260.67) and III and IV (D^2=174.30).

Since maximum genetic recombination and variation in the subsequent generation is expected from crosses that involve parents from the clusters characterized by maximum distances, crosses between genotypes selected from cluster V with cluster III, cluster II with cluster V and cluster III with cluster IV are expected to produce relatively better genetic recombination and segregation in their progenies. However, the selection of parents should consider special advantages of each cluster and each genotype within a cluster depending on the specific objective of hybridization program. Crosses involving genotypes belonging to most divergent clusters distances could be used for hybridization program to obtain good manifestations of heterosis and wide variability (Singh and Chaudhary 1987).

Table 10 Inter cluster genetic divergence (D2) based on 19 significant quantitative traits

Clusters	I	II	III	IV	V
I		24.57	87.47**	23.62	139.13**
II			34.48*	87.38**	260.67**
III				174.30**	371.29**
IV					53.17**
V					

**=Highly significant, (p<0.01) c^2=34.80, (p<0.05) c^2=28.87

4.8.5 Inter cluster distance (D2) analysis based on qualitative characters

All clusters showed a highly significant (P<0.01) difference among each other (Table 11). The highest inter-cluster distance (D^2=254.80) was obtained between clusters II and VI, followed by between clusters V and VI (D^2=212.05), between I and VI (D^2=188.70) and between cluster III and VI (D^2=162.25).

According to this results, maximum genetic recombination and variation in the subsequent generation is expected from crosses that involve parents from the clusters characterized by maximum distances, crosses between genotypes selected from cluster II with cluster V, cluster V with cluster VI, cluster I with cluster VI and cluster III with VI are expected to produce relatively better genetic recombination and segregation in their progenies. However, the selection of parents should be made based on special advantages of each cluster and each genotype within a cluster depending on the specific objective of hybridization program.

Table 11 Inter cluster Genetic divergence (D2) based on ten qualitative traits

Cluster	I	II	III	IV	V	VI
I		59.71**	106.45**	60.96**	59.34**	188.70**
II			158.24**	122.24**	21.79**	254.80**
III				99.27**	162.25**	72.85**
IV					68.67**	80.90**
V						212.05**
VI						

**=Highly significant, ($p<0.01$) $c^2=21.67$, ($p<0.05$) $c^2=16.92$

4.9 Principal Component Analysis

The principal component (PC) analysis (Table 12) showed that six principal components (PCs) i.e., PC1, PC2, PC3, PC4, PC5 and PC6 exhibited more than one Eigen value (4.431, 3.581, 2.511, 1.710, 1.424 and 1.110, respectively) and accounted for 77.7% of the total variation. Hence, these six PCs were considered important for further explanation. The first principal component PC1 explained 23.32% of the total variation, followed by PC2 (18.85%), PC3 (13.22%), PC4 (8.98%), PC5 (7.49%) and PC6 (5.84%). The first two principal components (PC1 and PC2) with values of 23.32% and 18.85%, respectively contributed more to the total variation (Table 13). Principal component analysis (PCA) is the most frequently used multivariate method (Crossa, 1990; Purchase, 1997). According to Chahal and Gosal, (2002) characters with the largest absolute values closer to unity within the first principal component influence the clustering more than those with lower absolute values closer to zero. Therefore, hundred bean weight, bean length, bean width, bean thickness, fruit length and plant height had more contribution to the total variation and they were the ones that most differentiated the clusters. Hence, these traits played

major role in classifying coffee accessions into different clusters and should be considered in selecting diverse parents in crossing program.

However, in this study, differentiation of the accessions into different clusters was because of a cumulative effect of a number of characters rather than the contribution of specific few characters. Characters having relatively higher influence in the principal component (PC1), like hundred bean weight, bean length, bean width, bean thickness, fruit length, plant height, stem diameter and average internodes length on main stem had more contribution to the total variation and were the ones that most differentiated the clusters. Variation in the second principal component (PC2) was mainly influenced by clean coffee yield, number of primary branch, number of bearing primary branch, length of longest primary branch, fruit width, hundred bean weight, canopy diameter and stem diameter. Traits such as average internodes length, number of primary branches, leaf width and leaf size were the major contributors of the variations in the third principal component (PC3). Variation in the fourth principal component (PC4) was mainly due the influence of reaction to coffee berry disease, plant height, clean coffee yield and coffee leaf rust. The fifth principal component was predominantly influenced by coffee leaf rust, plant height, fruit width and canopy diameter. The six principal components which contributed less to the total variation as compared to former components were characterized by height up to first primary branch, stem diameter and fruit length.

Therefore, almost all characters contributed to the discrimination of the tested accessions. Characters, such as number of primary branch, number of bearing primary branch, clean coffee yield, canopy diameter, length of longest primary branch, plant height, stem diameter, reaction to coffee berry disease, hundred bean weight, bean length, fruit width, fruit length, average internodes length, coffee leaf rust, bean width, bean thickness, leaf width and leaf size had more contribution to the total variation and were the ones that most differentiated the clusters. The present study confirmed that the coffee accessions showed significant variations for the characters studied and it suggested that many opportunities for genetic improvement through selection and conservation of the genotypes for future utilization.

Similar works done by Kebede and Bellachew (2008); Olika *et al.* (2011b); Gessese *et al.* (2015) and Masreshaw (2018), grouping of genotypes using principal component analysis in Ethiopia. Yigzaw (2005) also reported characters contributing for variation among

coffee genotypes like inter-node lengths, tree height, canopy diameter, number of branches, bean and fruit characters. Likewise, Masreshaw, (2018) reported average inter-node length of primary branches, average length of primary branches, canopy diameter, fruit width, fruit thickness, bean width, bean thickness and hundred bean weight contributed more to variation among Yayo coffee accessions. This finding is also in agreement with this finding Olika *et al.*, (2011) who reported bean length, hundred bean weight and leaf width contributed to the variation among Limmu coffee accessions.

Table 12 Eigen values and Eigenvectors of the first five principal components (PCs) for 19 significant characters of 64 Coffee Arabica accessions at Awada (2017/18)

Character	Eigenvectors					
	Prin1	Prin2	Prin3	Prin4	Prin5	Prin6
Coffee bean yield (kg)	0.40	0.50	-0.28	-0.51	0.13	-0.08
Plant height (cm)	0.56	0.27	0.30	0.58	-0.33	-0.06
HUFPB(cm)	0.37	0.02	0.06	0.09	-0.14	0.47
Stem diameter (cm)	0.20	0.68	0.08	-0.02	0.35	0.43
Canopy diameter (cm)	0.36	0.72	0.17	-0.01	0.34	0.24
Internodes length (cm)	0.50	0.12	0.50	0.45	-0.30	-0.17
Number bearing of primary branch	0.47	0.51	-0.43	0.11	-0.09	-0.26
Number of primary branch	0.45	0.56	-0.50	0.05	-0.17	-0.21
Length of longest primary branch (cm)	0.48	0.54	0.16	0.27	0.30	-0.07
Coffee berry disease (%)	-0.37	-0.30	0.39	0.50	0.27	0.20
Coffee leaf rust (%)	-0.08	-0.26	-0.25	0.48	0.62	-0.30
Hundred bean weight (g)	0.63	-0.56	-0.32	-0.04	-0.12	0.05
Seed length (mm)	0.63	-0.46	0.03	-0.01	-0.27	0.27
Seed width (mm)	0.65	-0.43	-0.10	-0.02	0.13	-0.18
Seed thickness (mm)	0.72	-0.46	-0.07	0.01	0.25	-0.17
Fruit length (mm)	0.66	-0.25	-0.07	-0.08	-0.03	0.32
Fruit width (mm)	0.45	-0.51	-0.26	0.00	0.41	0.17
Leaf Width (cm)	0.33	-0.03	0.78	-0.38	0.14	-0.23
Leaf Size (cm^2)	0.34	-0.10	0.76	-0.42	0.10	-0.20
Eigenvalues	**4.43**	**3.58**	**2.51**	**1.71**	**1.42**	**1.11**
Difference	0.85	1.07	0.81	0.28	0.31	0.13
Percent of variation	**23.32**	**18.85**	**13.22**	**8.98**	**7.49**	**5.84**
Cumulative	0.23	0.42	0.55	0.64	0.72	0.78

4.10 Shannon-Weaver Diversity Indices

Shannon-Weaver diversity indices (H`) is used to compare phenotypic diversity among qualitative characters. A low H` indicates unbalanced frequency classes for an individual trait and lack of diversity for the trait. In this study Shannon-Weaver diversity values were variable among traits and ranged from 0.35 to 1.18 (Table 13). Traits such as, branching habit, fruit shape, growth habit, overall appearance and stem habit showed Shannon diversity values of 0.66, 1.18, 1.05, 0.87 and 0.54, respectively, and exhibited more percentages contribution to genetic variability compared to others. The overall mean of H' value of 0.70 confirmed the existence of some level of diversity among the accessions.

This indicates that the aforementioned qualitative traits contributed more to genetic the variation of the germplasm in this study. Masreshaw (2018) also reported more contribution of leaf tip color, and unlikely stipule shape, leaf shape and leaf apex shape on 64 Yayo coffee collections. The highest diversity index (H') was found for fruit color, young leaf tip color, stipule shape and leaf shape (Masreshaw. 2018). About 89.1 % of the tested coffee accessions had lanceolate leaf shape (Appendix Table 3). Generally the diversity indices of all evaluated traits were above 0.35, indicating the presence of adequate variability for these traits among evaluated accessions.

Table 13 Shannon-Weaver diversity indices for ten qualitative morphological characters of 64 evaluated C. arabica accessions

Plant traits	H'	H_{max}	(%) contributed to variation
Growth habit	**1.05**	1.10	**95.45**
stem habit	**0.54**	0.69	**78.26**
Branching Habit	**0.66**	0.69	**95.65**
angle of insertion	0.48	0.69	69.57
Leaf tip color	1.01	1.61	62.73
leaf shape	0.35	0.69	50.72
leaf apex shape	0.38	0.69	55.07
stipule shape	0.45	1.10	40.91
fruit shape	**1.18**	1.39	**84.89**
OVA	**0.87**	1.10	**79.09**
Overall mean of H'	**0.70**	0.98	**71.23**

5 SUMMARY AND CONCLUSION

In this study, sixty four coffee accessions including six standard checks were evaluated in an 8X8 simple lattice design having two replications with eight genotypes per each incomplete block at Awada Agricultural Research Sub-Center. The objectives of the study were determining the extent of variability among *Coffee arabica* accessions, estimating association among coffee yield and morphological traits and, partitioning the correlation coefficients into direct and indirect effects and grouping of Amaro coffee accessions into different class based on quantitative and qualitative traits.

The analysis of variance showed the presence of significant differences for most of the measured quantitative characters considered, indicating the existence of variability among the tested accessions. Phenotypic variance was higher than the genotypic variances for all the characters indicating the influence of the environmental factors on these traits. For most of the traits genotypic coefficients of variation were very close to their corresponding estimates of phenotypic coefficient of variation, suggesting the greater role of the genotype in the expression of these traits.

High heritability estimate was observed for stem diameter, coffee berry disease reaction, fruit length, coffee leaf rust reaction, coffee bean yield, bean width, number of primary branches, number of main stem nodes, height up to first primary branch, hundred bean weight, fruit width, length of longest primary branch, plant height and Canopy diameter. This suggests that these traits are primarily under genetic control and selection for them can be achieved through their phenotypic performance. High genetic advance as percent of means was observed for coffee berry disease, coffee leaf rust and coffee bean yield coupled with high heritability. This condition indicates that there is good opportunity to improve these traits using the tested accessions. The genotypic correlation coefficients were higher than the phenotypic correlation coefficients demonstrating that, the observed relation-ships among the various traits were due to genetic causes.

Cluster analysis using significant quantitative traits confirmed the presence of variation among Amaro coffee accessions and grouped 64 Amaro coffee accessions in into five clusters. The smallest inter-cluster distance (D^2=23.62) was observed between clusters I and IV while the highest (D^2=371.29) was between clusters III and V. According to ten qualitative characters, the tested coffee accessions were grouped into six distinct groups.

The smallest inter-cluster distance (D^2=21.79) was observed between clusters II and V while the highest (D^2=254.80) was between clusters II and VI. Since maximum genetic recombination and variation in the subsequent generation is expected from crosses that involve parents from the clusters characterized by maximum distances, crosses between genotypes selected from cluster II with cluster V, cluster V with cluster VI, cluster I with cluster VI and cluster III with VI are expected to produce relatively better genetic recombination and segregation in their progenies.

In principal components analysis the first two principal components PC1 and PC2 with values of 23.32% and 18.85%, respectively, contributed more to the total variation. Almost all characters were contributed to the discrimination of tested accessions. However, hundred bean weight, bean length, bean width, bean thickness, fruit length and plant height had more contribution to the total variation and they were the ones that most differentiated the clusters. Estimates of frequency distribution and Shanon Index based on qualitative traits such as: branching habit, fruit shape, growth habit, overall appearance and stem habit with Shanon diversity values (H') values reveals more contribution to genetic variability compared to others. Generally, the present study confirmed the existence of enormous genetic variability among Amaro coffee collections for various important morphological traits.

Generally, the existence of genetic variability and association among traits in the base population is a key resource for breeder through selection and cross breeding in coffee improvement program. The present study confirmed the existence of enormous genetic variability among Amaro coffee germplasm for various important morphological traits. Hence there is an opportunity to exploit these traits to develop varieties that perform better from Amaro Kele coffee germplasm for the future coffee improvement program. However, additional accessions with other traits of interest like: Aromatic intensity, Aromatic quality, Acidity, Astringency, Bitterness, Body and flavor should be studied over year and locations. Furthermore, in order to confirm the present encouraging result, the current findings must be further studied with physiological, quality and biochemical analysis with the support of advanced molecular techniques which provides immense potential to ensure effective utilization, conservation and development of improved varieties.

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7 APPENDIX

Appendix Table 1 Mean performance of clean coffee yield and yield related quantitative traits of Amaro coffee accession at Awada, Southern Ethiopia during 2017/18 cropping season

Accession	YLD	PH	HUFPB	SD	CD	AINL	NBPB	NPB	LLPB	CLR
AK1	444.5(2.6)	223.3	21.7	4.35	186.7	4.7	55.5	70.0	108.5	8.9(17.1)
AK2	2474.5(3.4)	210.0	24.2	5.85	211.5	4.4	52.0	70.0	118.5	9.7(17.4)
AK3	1620.0(3.2)	245.0	25.0	5.45	215.5	5.4	48.5	68.0	117.9	2.5(8.3)
AK4	522.0(2.7)	222.5	20.4	5.40	206.7	4.9	52.0	69.5	111.4	16.7(24.1)
AK5	1585.5(3.2)	225.0	20.9	4.70	203.8	5.0	53.5	70.0	116.9	13.3(21.1)
AK6	1026.5(3.0)	240.8	24.2	5.10	205.8	5.5	53.5	67.5	119.3	8.0(15.4)
AK7	780.0(2.8)	208.4	20.5	5.00	208.7	4.3	50.0	68.5	113.0	25.0(29.9)
AK8	494.5(2.7)	229.2	17.5	4.40	184.8	5.0	50.5	69.5	108.8	24.2(29.4)
AK9	1175.5(3.1)	239.2	26.2	5.90	215.0	5.1	55.5	69.5	125.9	3.6(10.7)
AK10	1932.5(3.3)	221.7	24.7	5.15	207.9	4.8	55.0	70.0	120.4	31.7(34.1)
AK11	425.5(2.6)	232.0	22.0	4.40	193.8	4.3	57.5	76.5	112.5	25.0(29.7)
AK12	1180.0(3.1)	233.3	24.9	4.70	214.6	4.7	54.5	71.5	113.7	19.2(24.1)
AK13	971.0(3.0)	231.0	20.7	5.05	202.5	5.0	50.0	69.0	119.2	22.5(28.3)
AK14	555.0(2.7)	256.2	23.3	5.15	197.5	5.6	54.0	70.0	118.7	4.3(12.0)
AK15	1768.0(3.2)	243.7	23.2	5.55	221.7	5.0	56.0	75.0	121.4	3.1(8.3)
AK16	1874.5(3.3)	220.0	23.0	5.60	204.2	4.6	56.0	72.0	111.0	6.4(12.9)
AK17	78.5(1.9)	215.0	20.5	4.40	190.8	4.7	41.0	58.0	111.5	25.0(29.5)
AK18	1068.5(2.9)	221.7	19.2	5.50	202.9	4.9	45.5	71.5	119.0	10.0(18.4)
AK19	425.5(2.5)	227.5	23.8	4.80	196.3	4.8	53.0	68.5	118.0	27.5(31.6)
AK20	792.5(2.9)	231.7	20.9	5.50	208.8	4.8	53.5	72.0	115.4	6.2(14.4)
AK21	1418.0(3.1)	227.5	25.0	5.60	221.7	4.9	48.0	68.5	118.7	8.4(16.7)
AK22	51.0(1.5)	235.9	22.5	4.85	204.2	5.1	39.5	56.5	115.5	24.2(29.3)
AK23	1545.0(3.2)	216.7	20.0	5.55	207.9	4.8	49.0	66.5	117.2	3.5(10.7)
AK24	342.5(2.5)	237.5	16.7	5.40	207.5	5.1	49.5	70.0	117.5	25.0(30.0)
AK25	830.0(2.8)	199.2	20.2	4.75	190.4	4.7	44.5	63.0	103.2	8.0(16.4)
AK26	979.5(2.9)	216.7	23.0	5.35	199.6	4.7	50.0	67.5	113.9	46.7(43.1)
AK27	709.0(2.7)	226.7	27.2	5.35	201.7	4.8	51.5	67.5	112.2	20.0(26.1)
AK28	1112.5(3.0)	225.9	25.0	5.30	207.5	4.9	48.0	70.0	116.5	3.9(10.8)
AK29	331.5(2.4)	222.7	19.2	5.80	213.8	4.7	46.0	59.5	114.9	14.2(20.9)
AK30	695.5(2.6)	233.3	27.7	5.15	206.3	4.8	53.5	70.0	125.0	25.030.0)
AK31	484.0(2.7)	240.8	21.7	4.80	192.5	4.9	51.0	72.5	115.4	25.0(28.5)
AK32	879.5(2.7)	229.2	20.8	5.55	205.4	4.8	51.0	70.0	112.9	5.0(12.1)
AK33	874.5(2.9)	190.8	28.5	4.70	198.3	3.6	50.5	69.0	106.7	12.4(16.8)
AK34	1394.0(3.1)	243.4	25.0	4.60	188.4	5.5	49.5	66.0	112.5	6.4(14.6)
AK35	1449.0(3.2)	235.4	25.5	5.75	215.8	4.8	52.0	70.5	118.5	4.4(11.5)
AK36	925.5(2.9)	238.4	25.8	4.65	185.5	5.1	53.5	66.5	108.3	20.09(25.1)
AK37	787.0(2.9)	239.2	29.0	5.20	207.1	4.9	50.5	71.0	116.5	10.59(18.6)
AK38	1729.5(3.2)	217.5	25.3	5.20	210.9	4.2	53.5	72.5	117.5	17.6(24.3)
AK39	709.5(2.9)	200.9	23.5	4.90	190.0	4.2	45.0	64.0	98.4	15.9(23.4)
AK40	476.5(2.7)	252.7	31.0	5.50	220.0	5.4	49.5	65.5	113.5	10.49(15.6)
AK41	1093.0(3.0)	235.0	25.0	5.05	211.7	5.1	47.5	69.5	116.0	9.2(17.5)
AK42	190.5(2.2)	242.0	29.7	4.85	191.3	5.2	43.0	62.0	111.7	7.5(14.4)
AK43	24.0(1.1)	213.4	23.4	4.45	190.9	4.6	38.5	52.0	115.0	10.8(20.6)
AK44	476.5(2.7)	244.2	28.3	4.90	194.2	5.2	46.5	70.0	111.0	11.7(19.5)
AK45	983.0(3.0)	230.9	24.5	5.60	212.1	4.9	51.5	69.5	117.5	7.5(14.4)
AK46	1005.5(2.8)	230.0	19.2	5.05	203.6	5.1	51.0	68.5	114.4	7.5(15.7)
AK47	1433.0(3.0)	225.8	23.4	5.05	196.1	4.7	54.0	72.5	110.0	6.7(10.0)
AK48	698.5(2.8)	223.4	23.4	5.05	194.2	4.7	48.5	67.0	109.9	3.4(10.9)
AK49	1347.5(3.1)	261.7	28.3	5.60	212.5	5.7	53.0	72.0	119.7	20.9(27.0)

Accession	YLD	PH	HUFPB	SD	CD	AINL	NBPB	NPB	LLPB	CLR
AK50	1111.5(3.0)	243.3	25.5	5.75	218.0	5.1	55.5	72.0	120.7	21.7(27.3)
AK51	1297.0(3.1)	218.4	20.5	4.70	198.4	5.0	49.0	66.0	113.9	27.5(31.4)
AK52	900.0(3.0)	255.0	24.0	4.20	197.9	5.4	51.5	70.0	115.5	3.8(11.2)
AK53	739.5(2.9)	266.7	25.5	5.00	215.4	5.6	52.5	72.0	122.7	2.7(9.2)
AK54	1044.5(3.0)	237.5	24.2	5.30	203.3	5.2	56.5	70.0	119.2	35.8(36.7)
AK55	1106.0(3.0)	236.7	24.4	5.15	212.6	5.0	52.5	70.5	119.0	14.2(22.1)
AK56	1040.5(3.0)	243.4	22.5	4.65	208.1	5.3	48.0	68.5	120.3	30.0(33.2)
AK57	984.0(3.0)	224.2	23.4	5.55	223.8	4.7	52.0	71.0	119.0	6.0(14.2)
AK58	1237.5(3.0)	230.0	22.9	5.20	215.5	4.9	49.5	70.0	116.2	11.4(19.1)
1377	1111.5(3.0)	250.8	28.3	4.80	207.1	5.1	52.0	73.0	112.2	6.7(14.9)
971	1221.0(3.1)	207.0	24.3	4.60	194.2	4.4	47.5	67.5	116.8	14.2(22.1)
85257	1260.0(3.1)	226.7	17.5	4.95	205.0	5.0	53.5	70.0	115.2	5.0(12.9)
974	539.0(2.7)	213.3	24.2	4.85	186.7	4.5	41.5	62.5	105.3	6.9(15.1)
74112	382.5(2.6)	214.2	20.0	4.45	191.7	5.0	45.0	62.0	114.9	10.0(17.9)
7440	1146.5(3.0)	216.7	25.7	4.25	195.0	4.7	46.0	63.0	108.0	7.2(15.5)
Mean	957.7(2.8)	229.6	23.5	5.08	203.6	4.9	50.3	68.3	115.0	13.8(20.2
CV	34.06	5.84	9.82	4.82	4.74	7.6	8.22	5.12	3.97	27.45
Pvalue	<0.0001	0.0018	<0.0001	<0.0001	0.0049	0.017	0.0026	0.034	0.002	<0.0001
LSD(1%)	1236.06	38.18	6.14	0.70	25.66	1.04	10.45	12.87	13.06	18.45
LSD(5%)	926.87	28.63	4.62	0.53	19.30	0.78	7.84	9.68	9.79	13.84

Appendix Table 1 (Continued)

Accession	CBD	HBW	BL	BW	BT	FL	FW	LW	LS
AK1	27.5(30.3)	15.0	10.18	6.19	3.50	15.90	10.15	5.20	40.0
AK2	7.5(11.4)	12.8	9.34	6.16	3.59	15.05	10.70	5.35	43.4
AK3	1.7(7.4)	15.0	9.98	6.24	3.72	15.50	11.15	5.65	48.4
AK4	50.0(45.0)	13.0	9.62	6.34	3.53	14.40	10.35	5.40	42.0
AK5	10.9(18.9)	14.7	9.86	6.53	3.68	15.75	10.70	5.55	46.2
AK6	32.5(33.2)	15.6	10.78	6.12	3.74	16.35	10.45	5.65	47.9
AK7	35.0(36.3)	12.8	9.43	6.28	3.59	14.70	11.00	5.20	41.3
AK8	19.2(25.0	14.8	10.12	6.36	3.70	14.65	11.00	5.25	41.2
AK9	4.2(11.2)	12.9	9.79	6.52	3.69	15.05	11.15	5.50	44.7
AK10	1.7(5.3)	15.7	10.06	6.52	3.79	15.80	11.90	5.15	42.1
AK11	2.5(9.0)	14.2	9.43	6.16	3.63	15.35	11.45	4.95	39.2
AK12	3.9(10.4)	15.1	9.74	6.57	3.69	14.20	11.10	5.30	41.8
AK13	19.2(25.3)	14.8	9.88	6.30	3.56	15.00	11.40	5.00	39.0
AK14	17.5(23.6)	16.2	10.78	6.48	3.86	16.25	11.30	5.10	40.7
AK15	7.0(14.0)	13.7	9.62	6.11	3.41	14.60	10.55	5.20	41.2
AK16	0.9(3.7)	13.7	9.88	6.35	3.64	15.30	11.00	5.40	43.3
AK17	62.2(54.3)	15.0	9.91	6.32	3.85	14.65	11.50	5.35	42.9
AK18	2.5(6.5)	13.2	9.11	5.47	3.11	14.10	10.40	5.00	37.3
AK19	85.0(67.2)	14.5	10.35	6.51	4.17	15.10	10.70	5.45	44.3
AK20	2.5(9.0)	11.3	9.39	6.20	3.58	15.05	11.60	5.65	46.1
AK21	1.7(5.3)	13.8	9.74	6.16	3.80	15.30	10.95	5.40	45.6
AK22	79.0(63.8)	11.2	8.54	6.01	3.40	14.65	11.30	5.35	42.9
AK23	3.4(7.5)	13.2	9.45	6.41	3.70	14.70	11.10	5.60	45.5
AK24	75.9(61.0)	16.0	10.42	6.44	3.89	15.10	11.25	5.45	43.5
AK25	15.9(20.3)	13.3	9.48	6.26	3.65	14.50	11.00	5.00	39.2
AK26	40.8(39.0)	17.2	10.27	6.67	4.09	16.75	12.15	5.50	45.7
AK27	54.2(47.4)	15.8	10.96	6.52	4.01	17.10	11.75	5.15	41.5
AK28	9.7(17.8)	19.3	11.32	6.48	3.78	17.40	12.20	5.35	43.7
AK29	51.7(46.0)	12.7	9.96	6.17	3.48	15.60	11.70	5.10	38.7

Accession	CBD	HBW	BL	BW	BT	FL	FW	LW	LS
AK30	3.5(7.7)	13.4	9.47	6.35	3.84	14.05	11.10	4.95	39.3
AK31	28.3(31.3)	14.5	9.36	6.18	3.67	15.00	11.55	5.10	38.9
AK32	9.2(17.6)	13.7	9.78	6.40	3.50	15.30	10.85	5.30	41.0
AK33	2.7(6.7)	16.3	9.99	6.73	3.78	14.75	11.75	4.95	37.5
AK34	10.0(18.2)	14.9	10.05	6.90	3.78	15.10	11.65	5.35	43.5
AK35	11.7(19.1)	13.4	9.43	6.53	3.67	14.60	11.25	5.15	39.5
AK36	21.7(27.1)	19.2	9.83	6.70	3.83	15.40	11.75	4.90	38.4
AK37	16.7(21.9)	14.6	9.78	6.00	3.47	14.95	10.65	4.85	36.0
AK38	21.7(27.5)	13.3	9.67	6.20	3.64	14.60	10.95	5.20	41.1
AK39	45.9(42.5)	12.1	9.76	6.04	3.10	14.55	10.90	5.00	38.8
AK40	79.2(62.9)	12.2	9.80	6.42	3.70	14.40	10.65	5.90	49.1
AK41	10.0(16.4)	14.3	9.53	6.64	3.55	14.65	10.95	5.60	45.0
AK42	44.7(41.9)	16.5	10.66	6.82	3.83	15.25	11.30	5.30	42.6
AK43	61.7(54.6)	12.5	9.83	6.30	3.41	14.55	10.95	5.70	48.9
AK44	24.5(28.4)	13.3	9.85	6.44	3.54	14.70	10.65	5.40	44.3
AK45	15.2(18.3)	14.4	9.99	6.56	3.66	15.30	10.80	5.30	41.7
AK46	5.0(12.1)	14.0	9.73	6.35	3.74	15.40	10.80	5.50	44.4
AK47	0.3(3.3)	14.3	10.16	6.41	3.76	14.90	10.95	5.40	43.9
AK48	1.7(5.3)	13.6	9.60	6.35	3.69	15.05	10.70	5.40	43.0
AK49	1.7(5.3)	15.8	10.50	6.85	4.02	16.50	12.25	5.65	44.9
AK50	7.5(11.4)	14.7	10.06	6.33	3.82	16.40	11.35	5.55	44.7
AK51	10.9(13.9)	13.8	9.63	6.53	3.73	14.00	10.95	5.45	43.3
AK52	3.4(7.5)	16.5	10.45	6.60	3.87	15.70	11.00	5.30	42.9
AK53	0.9(3.7)	15.7	10.40	6.54	3.72	15.60	11.20	5.35	43.4
AK54	15.0(22.5)	14.4	9.59	6.77	3.72	14.85	11.05	5.20	41.3
AK55	14.2(16.1)	14.2	10.01	6.47	3.69	18.20	10.95	5.30	41.3
AK56	4.7(12.4)	15.8	9.95	7.13	4.10	15.40	12.05	5.35	41.1
AK57	3.3(10.5)	14.8	9.89	6.38	3.67	15.90	11.05	5.55	44.9
AK58	3.4(10.2)	15.6	9.94	6.81	4.01	15.45	11.50	5.20	39.9
1377	0.0(0.0)	15.9	10.33	6.73	4.08	16.00	11.30	5.65	47.5
971	0.0(0.0)	19.8	10.10	7.02	4.30	16.30	12.30	5.60	45.8
85257	0.0(0.0)	17.7	10.39	6.96	3.95	16.55	11.45	5.45	44.8
974	0.0(0.0)	17.3	10.44	6.51	3.88	15.85	11.85	5.35	42.9
74112	0.0(0.0)	13.7	9.75	6.20	3.64	14.40	10.95	5.75	48.9
7440	0.0(0.0)	15.5	10.56	6.32	3.73	16.10	11.40	5.60	48.2
Mean	18.8(20.6)	14.6	9.93	6.42	3.71	15.31	11.18	5.34	42.8
CV(%)	50.33	9.75	5.2	3.1	5.27	3.31	3.3	3.85	6.61
Pvalue	<0.0001	0.0001	0.0293	<0.0001	0.0022	<0.0001	0.0007	0.0086	0.0111
LSD(1%)	29.18	4.14	1.37	0.52	0.52	1.44	1.05	0.55	7.52
LSD(5%)	29.88	3.11	1.03	0.39	0.39	1.08	0.78	0.41	5.66

Values in the brackets indicate transformed mean

Appendix Table 2 Summary of standard checks performance for coffee berry at Awada, Leku and Wonago

Variety	Awada (1738m a.s,l)		Leku	Wonago(1886m a.s,l)	
	August 2017	August 2018	August 2018	August 2017	August 2018
1377	0.0	0.5	0.0	2.50	11.00
971	0.0	0.0	0.0	0.06	0.08
974	0.0	0.0	0.0	0.10	4.34
85257	0.0	0.0		0.08	0.08
74112	0.0	0.0		0.03	0.06
7440	0.0	0.0		0.00	0.10

Appendix Table 3 Test for Normality of residuals in each of the separate ANOVA modelusing the Shapiro-Wilk (W) statistic

Traits	Value of W	P-value=Pr<W	Traits	Value of W	P-value=Pr<W
PH	0.993329	0.8088	AINLPB	0.980305	0.0594
HUFPB	0.98038	0.0604	FL	0.97967	0.0514
SD	0.98515	0.1774	FW	0.988492	0.3623
LLPB	0.980845	0.0671	FT	0.98311	0.1122
NPB	0.980435	0.0611	LL	0.980853	0.0672
NBPB	0.981227	0.0732	LW	0.979911	0.0543
NMSN	0.979873	0.0538	LS	0.994544	0.9075
AINL	0.984714	0.1610	HBW	0.96788	0.0553
CD	0.986774	0.2530	SL	0.991932	0.6722
ALPB	0.984337	0.1480	SW	0.97982	0.0532
NNPB	0.990059	0.4908	ST	0.988286	0.3475

Appendix Table 4 Observable phenotypes (%) for ten qualitative morphological characters of 64 evaluated C. arabica accessions

Plant traits	Observable phenotypes	Percentage
Growth habit	Open	**35.90**
	Intermediate	**43.80**
	Compact	**20.30**
stem habit	Stiff	23.40
	Flexible	76.60
Branching Habit	Many branches (primary) with few secondary branches	37.50
	Many branches (primary) with many secondary branches	62.50
angle of insertion	Spreading	18.80
	semi-erect	81.30
Leaf tip color	light green	**1.56**
	Green	**14.10**
	Bronze	**67.20**
	light bronze	**12.50**
	redish bronze	**4.69**
leaf shape	Ovate	10.90
	Lanceolate	89.10
leaf apex shape	Acuminate	12.50
	Apiculate	87.50
stipule shape	Ovate	87.50
	Triangular	3.13
	Deltate	9.38
fuit shape	Roundish	**28.10**
	Obovate	**6.25**
	Elliptic	**48.40**
	Oblong	**17.20**
OVA	elongated conical	28.10
	Pyramidal	62.50
	Bushy	9.38

Pruning (stumped) trial

RI

Incomplete Block = 1									Incomplete Block = 2									Incomplete Block = 3									Incomplete Block = 4							
1	2	3	4	5	6	7	8		9	10	11	12	13	14	15	16		17	18	19	20	21	22	23	24		25	26	27	28	29	30	31	32
*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*
*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*
*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*
*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*
*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*
*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*
Incomplete Block = 8									Incomplete Block = 7									Incomplete Block = 6									Incomplete Block = 5							
64	63	62	61	60	59	58	57		56	55	54	53	52	51	50	49		48	47	46	45	44	43	42	41		40	39	38	37	36	35	34	33
*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*
*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*
*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*
*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*
*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*
*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*

RII

Incomplete Block = 1									Incomplete Block = 2									Incomplete Block = 3									Incomplete Block = 4							
65	66	67	68	69	70	71	72		73	74	75	76	77	78	79	80		81	82	83	84	85	86	87	88		89	90	91	92	93	94	95	96
*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*
*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*
*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*
*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*
*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*
*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*
Incomplete Block = 8									Incomplete Block = 7									Incomplete Block = 6									Incomplete Block = 5							
128	127	126	125	124	123	122	121		120	119	118	117	116	115	114	113		112	111	110	109	108	107	106	105		104	103	102	101	100	99	98	97
*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*
*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*
*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*
*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*
*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*
*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*

Figure 3Field layout for simple lattice design in the study site

Crossing block

Number of treatments=58 indigenous Amaro coffee and 6 checks

Number of tree per plot = 6, Spacing between coffee trees = 2m * 2m

Spacing between replication = 4m,   Spacing between incomplete block = 3m